TREATMENT AND BENEFICIATION OF KRAFT MILL SLUDGE TO HYDROGEN AND METHANE FOR BIOENERGY PRODUCTION

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by

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As the candidate's supervisor, I have approved the submission of this thesis

Signed: _____________ ____

PREFACE

The research contained in this thesis was conducted by the candidate while based in the Discipline of Chemical Engineering, School of Engineering, of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Howard Campus, Durban, South Africa, under the supervision of Prof. B. B. Sithole and the co-supervision of Prof. E. B. G. Kana and Dr P. Lekha. The research was financially supported by the Council for Scientific and Industrial Research (CSIR) and the Department of Science and Innovation (DSI) Waste Roadmap.

The contents of this work have not been submitted in any form to another tertiary institution and, except where the work of others is acknowledged in the text, results reported are due to investigations by the candidate.

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DECLARATION 1: PLAGIARISM

I, Daneal Carmine Solange Rorke, declare that;

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then;
    a. Their words have been re-written, but the general information attributed to them has been referenced
    b. Where their exact words have been used, then their writing has been placed in italics and inside quotation marks and referenced
5. Where I have used material for which publications followed, I have indicated in detail my role in the work
6. This dissertation is primarily a collection of material, prepared by myself, published as journal articles. In some cases, additional material may be included
7. This thesis does not contain text, graphics or tables copied and pasted from the internet, unless specifically acknowledged, and the source being detailed in the thesis and in the references sections.

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Date: 17 November 2021

DECLARATION II: PUBLICATIONS

This thesis involves a compilation of manuscripts. Each chapter is an individual entity, prepared as per the journals' specifications; hence, some repetition between chapters is inevitable. The first author (student) conducted all experimental work, data collection and manuscript preparation, guided by the second, third and fourth (supervisor) author. The asterisk (*) indicates the corresponding author.

1. Rorke, D. C. S*., Lekha, P., Gueguim Kana, E. B. and Bruce Sithole, B. Beneficiation of paper mill sludge: Current strategies. In the process of submission. (Chapter 2)
2. Rorke, D. C. S*., Lekha, P., Kana, E. B. G. and Sithole, B. B. (2021). Surfactant-assisted green liquor dregs pretreatment to enhance the digestibility of paper mill sludge. Published in: *International Journal of Hydrogen Energy 46*(41): 21359-1371. (Chapter 3)
3. Rorke, D. C. S*., Lekha, P., Kana, E. B. G. and Sithole, B. B. Effect of pharmaceutical wastewater as nitrogen source on the optimisation of simultaneous saccharification and fermentation hydrogen production from paper mill sludge. Submitted and under review in *Sustainable Chemistry & Pharmacy*. (Chapter 4)
4. Rorke, D. C. S*., Lekha, P., Kana, E. B. G. and Sithole, B. B. Enhanced methane potential from two-stage anaerobic co-digestion of paper mill sludge and pharmaceutical wastewater. Submitted and under review in *Current Research in Green and Sustainable Chemistry*. (Chapter 5)

Conference Presentations:

1. Rorke, D. Lekha, P., Gueguim Kana, E. B., Sithole, B. B. Valorisation of pulp and paper mill sludge for biofuel production. School of Life Sciences Annual Postgraduate Research Day, Westville Campus, 22 May 2019. Poster presentation

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ABSTRACT

The key drivers behind research into renewable and sustainable practices are escalating global energy demands and financial instability linked to the depletion of fossil fuel-derived energy resources. Additional drivers include the environmental ramifications of continued fossil fuel usage and unsustainable industrial waste disposal methods. As the fifth-largest global coal producer, South Africa is heavily dependent on coal for its energy needs, accounting for approximately 80% of its greenhouse gas (GHG) emissions in 2015. Efforts to decarbonise the economy and promote low-carbon technologies have thus been on the rise. Examples include implementing a carbon tax, sectoral GHG emission targets, carbon budgets and phasing out inefficient fossil fuel incentives.

The South African pulp and paper industry plays an essential role in the economy by contributing 4% and 25% to the country's manufacturing and agricultural GDP, respectively. In 2019, 4.1 million tonnes of pulp and paper products were produced in South Africa. As a water-intensive industry, advanced water treatment and recycling technologies are implemented, generating significant solid waste volumes as paper mill sludge (PMS) of up to 100 tonnes per 550 tonnes of pulp produced. The industry relies heavily on landfilling as its primary waste disposal method, with up to 69% of the generated PMS being landfilled. Due to the environmental concerns of landfilling, such as GHG emissions, leaching of toxic compounds into the surrounding land and water and limited land space availability, environmental regulations have become stricter, impeding the future applicability of landfilling. Biorefineries have gained significant interest as an approach to utilise biomass such as industrial wastes efficiently. Fuels, chemicals, energy and heat can be produced from industrial waste streams, adding value to what many industries consider a financial and environmental burden.

Therefore, the beneficiation of PMS can contribute to the circular economy by serving as a low-cost, abundantly available feedstock for sustainable energy generation. The bioproduction of hydrogen and methane from PMS as alternative energy carriers offers a sustainable approach to energy production. Hydrogen is a CO_2-neutral energy source with a high energy content that can be converted to electricity in fuel cells, producing only water when combusted. In comparison, methane exhibits an octane rating higher than gasoline and produces less CO_2 than fossil fuels upon combustion. Furthermore, the application of biohythane, an advanced fuel mixture of hydrogen and methane, shows great potential to reduce GHG emissions and improve engine combustion yield in the automotive industry. Unfortunately, the presence of process rejects, ash, and residual lignin in PMS waste streams further contributes to its recalcitrance to microbial degradation, which leads to low product yields and high process times, thus significantly hampering the applicability of its beneficiation.

Therefore, this study aimed to develop economic beneficiation strategies to enhance the amenability of PMS to microbial digestion and improve subsequent process efficiency of hydrogen and methane production. A combined mixture and factorial Response Surface Methodology (RSM) design was used to develop a novel pretreatment protocol using green liquor dregs (GLD), a waste generated by the pulp and paper industry, to improve the enzymatic hydrolysis of PMS. Optimised process conditions of 56% GLD: 44% PMS, 4.5% Tween-80, 60 min heating time, and a solid-to-liquid ratio (S:L) of 9.5% gave a maximal reducing sugar release of 16.38 g/L. Elemental analysis of each phase of the pretreatment protocol also showed a significant reduction in the concentrations of heavy metals; aluminium (81.39%), chromium

(74.05%), cobalt (83.66%), nickel (88.06%), cadmium (88.89%), tin (83.82%) and lead (75.24%), and the complete removal of mercury. This is the first account of the utilisation of GLD as a pretreatment agent for PMS. Thereafter, the use of pharmaceutical wastewater (PW) as a supplementary nitrogen source to balance the system's carbon-to-nitrogen ratio (C/N) in the optimisation of simultaneous saccharification and fermentation (SSF) hydrogen production from pretreated PMS was assessed, using a Box-Behnken design. The investigated process parameters included nitrogen source, enzyme dosage, substrate concentration, and pH. Using PW as the nitrogen source gave a 2.26 and 39.38% increase in the optimised hydrogen yield, compared to yeast extract and ammonium nitrate, respectively. A maximum hydrogen yield of 33.56 mL/g volatile solids $(VS)_{added}$ was obtained. Kinetic studies using the modified Gompertz model produced comparable data between PW and yeast extract, displaying a 3.26 h difference in process lag time. Furthermore, the maximum potential hydrogen production rate (R_m) obtained using PW was 9.22 mL/h, exhibiting an 8.73% increase compared to ammonium nitrate.

The feasibility of using the effluent generated from the optimised processes for a two-stage anaerobic digestion (TSAD) system for methane production was then determined and compared to conventional single-stage anaerobic digestion (SSAD) of pretreated PMS. A low methane yield of 4.79 mL/gVS_{added} was obtained using SSAD. A significant improvement in the methane yield was observed using TSAD, reaching 30.88 mL/gVS_{added}. However, re-adjustment of the effluent's C/N to 25 increased the process duration from 10 days to 17 days and negatively impacted the methane yield, resulting in an 8.16% reduction. In addition, a significant variation in process kinetics was observed between PW and yeast extract in the SSAD system, illustrated by production rates (R_m) of 0.29 mL/h for PW and 1.29 mL/h for yeast extract. The TSAD system exhibited enhanced stability for both nitrogen sources, exhibited by PW and yeast extract supplemented processes giving maximum potential gas production volumes (G_m), maximum potential gas production rates (R_m) and lag times (t_L) of 113.28 and 104.93 mL, 1.28 and 1.40 mL/h and 4.25 and 1.71 h, respectively. Although C/N adjustment showed higher G_m values of 119.70 and 135.89 mL for PW and yeast extract, respectively, production rates were significantly reduced (0.50 mL/h). Compositional analysis of the resultant digestate showed a high VS content and C/N ratio, and an approximate NPK ratio of 2.3:1:2 from SSAD, whereas TSAD digestate exhibited an NPK ratio of 4.8:1:2, presenting almost double the nitrogen content. A high electrical conductivity (EC) value of 18.50 mS/cm observed in the digestate of TSAD was indicative of high sodium levels from PW, illustrating that, if the effluent C/N is adjusted to 25, the digestate of TSAD would require dilution before use as a soil amendment.

To the author's best knowledge, this is the first study describing the optimal beneficiation of PMS from the South African pulp and paper industry by pretreatment and subsequent two-stage anaerobic digestion for the production of hydrogen and methane. This study demonstrated the potential of PMS as a feedstock for bioenergy production, as well as the effects of supplementing its nitrogen deficiency with a secondary waste stream such as PW on process efficiency. Incorporating industrial wastes such as GLD and PW as alternatives to reagents such as NaOH and yeast extract to supplement fermentation processes can contribute to the circular economy concept by recycling waste streams. This improves the economic feasibility of PMS beneficiation and creates a sustainable energy solution to the disposal of PMS.

This study has generated; one paper that has been published in a high impact, peer-reviewed journal, two papers that have been submitted for publication, and one more paper that is currently being prepared for submission to a peer-reviewed journal.

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CHAPTER ONE

General Introduction

1.1 Waste generation in the pulp and paper industry

The pulp and paper industry is one of the largest global industries in which at least 400 million metric tonnes of paper and cardboard are produced per annum (Statista, 2021). Specifically, the South African pulp and paper industry produced 2.16 million tonnes of paper, tissue and packaging products and 1.94 million tonnes of pulp in 2019, contributing more than R24 billion to the country's gross domestic product (GDP) (PAMSA, 2019). The industry is both energy and water-intensive, requiring large volumes of water, between 50 and 60 m^3/tonne paper produced (Ram et al., 2020), with lower levels of water recycling in developing countries leading to increased production of process wastewater (Toczyłowska-Mamińska, 2017). Water treatment and recycling protocols within the pulp and paper industry contribute significantly to the solid waste generated, which includes inorganic materials such as ash, green liquor dregs, lime mud and slaker grits (Simão et al., 2018) and paper mill sludge (PMS) as the predominant organic portion (Coda et al., 2002). At approximately 100 tonnes of sludge generated per 550 tonnes of pulp produced (Haile et al., 2021), an estimated 0.5 million dry tonnes of sludge is generated each year in South Africa (Boshoff et al., 2016) and includes both primary and secondary sludge generated from wastewater treatment within pulp and paper mills. The chemical composition of the sludge types is mainly dependent on the type of paper produced, the water treatment technique implemented and the type of materials used, resulting in sludge streams from different paper mills displaying significant variation (Kuokkanen et al., 2008).

Primary sludge generated from primary clarification of wastewater treatment makes up 70% of the sludge generated and contains carbohydrate-rich fibrous aggregates, fillers, ash, and low amounts of trace elements and heavy metals (Simão et al., 2018). The wastewater generated from primary clarification then undergoes a secondary biological treatment involving the microbial conversion of soluble organic matter to carbon dioxide (CO_2) and water. The resultant sludge consists of activated sludge, sludge from secondary decanters and thickeners and cake from the filter press, typically containing a higher nutrient content than primary sludge (Simão et al., 2018). These sludge types are often combined to reduce handling difficulty during treatment and disposal.

One of the main challenges associated with PMS disposal is the low solids content and, thus, the requirement of sludge reduction by dewatering, which often contributes to more than half of the wastewater treatment costs (Meyer et al., 2018). Simão et al. (2018) and Bajpai (2012) outlined a number of sludge reduction techniques such as dissolved air floatation clarification, centrifugation, belt pressing, filtration, heat treatment, chemical oxidation, and the use of membrane reactors. After dewatering, PMS is predominantly disposed of by landfilling, land-spreading or incineration (Haile et al., 2021), as illustrated in Figure 1.1.

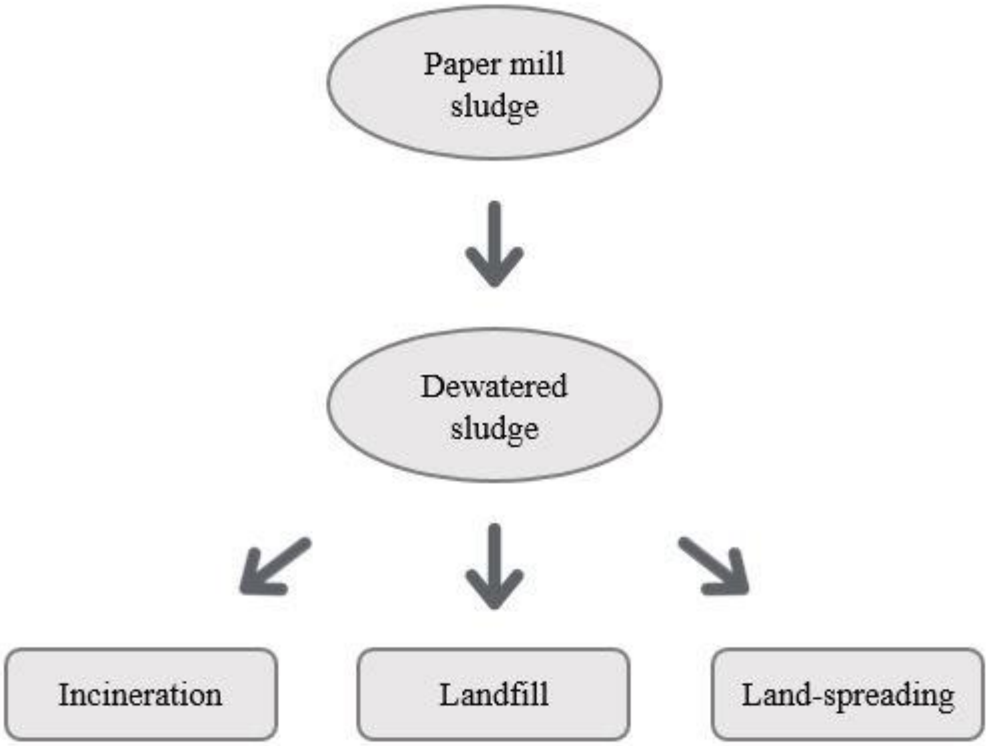

Figure 1.1: Common disposal methods of sludge generated from wastewater treatment in the pulp and paper industry.

Availability of landfill sites and low gate fees in South Africa make landfilling the primary disposal route for PMS. Up to 69% of the PMS generated is disposed of in landfills; however, significant quantities of greenhouse gases (GHGs) such as CO_2 and methane (CH_4) are released into the environment (Likon and Trebše, 2012). Additionally, the environmental impact of toxic compounds leaching into the soil and water, and odour production from pollutants poses a risk to the continued use of landfills for PMS disposal (Bajpai, 2012). In an effort to reduce the quantity of waste disposed of by landfilling and to increase recycling, South Africa introduced the National Environmental Management: Waste Act (Act No. 59 of 2008) to regulate waste management and monitor gas emissions, air, leachate and water quality (Herselman and Moodley, 2009). Subsequently, treatment and disposal of PMS are thus regulated by the *Waste Classification and Management Regulations*, *Norms and Standards for Assessment of Waste for Landfill Disposal* and *Norms and Standards for Disposal of Waste to Landfill* of the National Environmental Management (NEM) Waste Act No. 59 of 2008 (Department of Water and Environmental Affairs, 2013).

PMS can be incinerated on-site for energy recovery in a fluidised bed or circulating fluid bed combustion chamber, in bark boilers, by pyrolysis, gasification and wet oxidation, among others (Likon and Trebše, 2012). Although incineration allows for energy recovery, the main objective is to reduce the amount of PMS that is disposed of in landfills, achieving 80-90% volume reduction (Monte et al., 2009). However, the high moisture content of PMS and high capital investment reduces the economic feasibility of these energy-intensive methods (Bajpai, 2012), exhibited by PMS's low heating value of 15.4 to 15.8 MJ/kg (Duncan et al., 2020). In countries faced with decreased landfill space and high incineration energy requirements, land-spreading shows potential as a low-cost method of disposal (Bajpai, 2012). The use of PMS for land-spreading can improve both physical characteristics of soil and provide nutrients to rehabilitate degraded soil (Herselman and Moodley, 2009). Land-spreading has been successfully implemented as a replacement for manure in agriculture in Canada, with the heavy metal content of PMS being lower than that of cattle manure and municipal sludge (Bajpai, 2012). Landfilling and incineration exhibit numerous economic downfalls, including increasing landfill gate and transport fees and significant capital- and energy-intensive requirements. Interest remains in developing alternative end-of-life uses for PMS as it is carbohydrate-rich and is available in large amounts at a low cost.

1.2 The role of anaerobic digestion in economic decarbonisation

The global dependence on fossil fuel-derived energy and the environmental pollution associated with the combustion of fossil fuels has encouraged the drive for renewable energy production (Balat and Balat, 2009). In an effort to limit global warming to 1.5°C above pre-industrial levels, 196 parties, including South Africa, have adopted the 2015 Paris Agreement, a treaty on climate change. Parties involved are aiming to reach global GHG emission peaking as soon as possible to achieve climate neutrality (UNFCCC, 2015). In South Africa, more than 90% of the country's energy is generated from coal (Department of Environmental Affairs, 2020). Up to 37% of the energy demand is from the country's industrial sector (Department of Energy, 2016), illustrating the significant dependence of the country and its industries on fossil-fuel generated energy. To promote low carbon development, South Africa has committed to identifying and reducing the harmful impacts of inefficient fossil fuel subsidies and incentives, and implemented a carbon tax of R120/tonne CO_2-eq and activity-specific carbon budgets specifying the maximum volume of emissions that various activities may emit over a determined period (Department of Environmental Affairs, 2020). Furthermore, to reduce the quantity of waste destined for landfills, South Africa has adopted the National Waste Management Strategy (NWMS), which follows the waste management hierarchy of waste avoidance and reduction, re-use, recycling, recovery, treatment and disposal.

Although the waste-to-energy concept is not the preferred waste management method in South Africa, anaerobic digestion (AD) of organic waste for energy generation has been successfully implemented in many European countries (Scarlat et al., 2018; Caposciutti et al., 2020). AD generates biogas, a mixture of CH_4, CO_2 and lower amounts of other gases, by microbial conversion under anaerobic conditions (Li et al., 2019). AD of wastes contributes to the circular economy concept and exhibits advantages such as the reduction of waste destined for landfills, reduction of GHGs from landfills, and provision of an alternative energy resource for industries by capturing the biogas produced (Caposciutti et al., 2020; Uddin et al., 2021). Furthermore, the use of waste materials for energy generation has the potential to significantly contribute to the decarbonisation of the economy, especially if economic feasibility and process efficiency are significantly improved (Ellacuriaga et al., 2021). In addition, upgrading the captured biogas to biomethane would allow for easier storage as a compressed gas or liquid after liquefaction (Caposciutti et al., 2020).

1.3 Paper mill sludge as a resource for biogas production

The production of biogas by the AD of lignocellulosic wastes has continued to gain interest. Several studies have assessed a wide range of lignocellulosic wastes for their potential to produce biogas, including rice straw (Amnuaycheewa et al., 2016), yard trimmings (Zhao et al., 2014), birch wood (Mulat et al., 2018) and corn straw (Fu et al., 2015). As a residual lignocellulosic waste, PMS is predominantly made up of cellulosic fibres and rejects from the papermaking process (Duncan et al., 2020). Although the pulping process reduces the recalcitrance of this lignocellulosic waste, the presence of inhibitory substances such as fillers, ash, and wood extractives reduces the potential release of fermentable carbohydrates during enzymatic hydrolysis (Gurram et al., 2015). Significant effort has been made in developing pretreatment techniques to increase the release of fermentable sugars from PMS (Veluchamy and Kalamdhad, 2017; Alkasrawi et al., 2021); however, incorporating pretreatment protocols that make use of chemicals often considerably reduces the economic feasibility.

Additionally, the high carbohydrate content of PMS results in a high carbon-to-nitrogen ratio (C/N), which severely hampers the process efficiency of AD (Priadi et al., 2014). To enhance AD efficiency, hydrogen can be produced by dark fermentation (DF), generating volatile fatty acids (VFAs), which can be converted to CH_4 during AD, and hydrogen gas. As the hydrogen production process exhibits low substrate conversion, PMS can undergo two digestion stages; to produce VFAs and hydrogen and methane and carbon dioxide from the effluent of hydrogen production. This provides increased process control and reduced digestion times (Martínez-Gutiérrez, 2018).

1.4 Problem statement

Considered one of the top 10 global coal producers, South Africa's primary source of GHG emissions stems from coal-derived electricity production. As of 2020, the country's net GHG emissions reached 512 million tonnes CO_2-eq (Department of Environmental Affairs, 2020). To reduce this, incorporating low-carbon, renewable energy sources should be considered (Fisher and Downes, 2015; Rennkamp and Marquard, 2017). Furthermore, almost 90% of the 42 million tonnes of the waste generated in South Africa is disposed of in landfills, with many of the available landfills almost at capacity, with less suitable space available to build new landfills (Department of Environmental Affairs, 2020). Even though landfill gate fees have increased from R167-R252/tonne to R419-R671/tonne due to landfill air spaces reaching near capacity, these fees remain relatively low (Godfrey and Oelofse, 2017). Landfill gate fees remain low in an effort to reduce illegal dumping; however, this severely hinders progress towards diversifying disposal methods.

The introduction of AD as a means of capturing gases emitted from waste degradation, in the form of biogas can significantly contribute to the decarbonisation of the South African economy by providing an alternative treatment and disposal route by recycling suitable organic wastes and reducing the waste destined for landfills (Uddin et al., 2021). However, for AD to significantly contribute to decarbonisation, process efficiency is of great significance, and appropriate process conditions must be established. Paper mill sludge is a lignocellulosic, solid, organic waste generated by the pulp and paper industry in large volumes. Up to 69% of the 0.5 million tonnes of sludge generated in South Africa is disposed of in landfills (Likon and Trebše, 2012; Boshoff et al., 2016). Along with paper process rejects, PMS contains up to 68% cellulose which can be converted to precursors for biogas production (Du et al., 2020). Diversion of this waste stream to AD offers a means to reduce its disposal in landfills and reduce GHG emissions associated with its disposal, promoting a circular economy (Abad et al., 2019). However, low product yields due to the presence of inhibitory compounds and slow-degradation time significantly hinder biogas production efficiency.

As one of the parties to have adopted the 2015 Paris Agreement, South Africa has committed to reducing the harmful impacts of inefficient fossil fuel subsidies and incentives. Two of South Africa's most challenging sources of GHG emissions are coal production and the country's heavy reliance on landfilling for waste disposal. Although many organic waste streams can be considered easily digestible through AD, many industrial waste streams contain trace amounts of chemical compounds that are inhibitory to AD, rendering the use of waste treatment by AD unfeasible. Alleviating the negative impacts of slow and inefficient AD processes, implementing a waste-based pretreatment protocol, followed by waste-supplemented hydrogen and methane production are the main motivations for the study.

The novelty of this study lies in the inclusion of low-cost waste materials such as alkaline waste streams generated from the pulp and paper industry for PMS pretreatment and a nutrient-rich waste stream to balance the nutritional and nitrogen deficiency of PMS during microbial processes, which can significantly reduce the costs associated with its valorisation. This can be achieved by enhancing process performance by modelling and optimising. Response Surface Methodology (RSM) can be used to identify and compute the complex interactions between process parameters. Furthermore, the incorporation of enzymatic hydrolysis during fermentation to improve product yields can be implemented. Assessing process dynamics using kinetic models such as the modified Gompertz model will also contribute to optimal process control, resulting in a higher quality product in a shorter process duration.

1.5 Aims and objectives

This study aimed to improve the amenability of PMS to microbial degradation using a waste-based pretreatment protocol. Additionally, the potential of PMS for improved hydrogen and methane production was investigated, using a secondary waste stream for nitrogen supplementation.

The following specific objectives were carried out to achieve the aim of the study;

(i) Develop a pretreatment protocol to enhance the fermentable sugar release and, thus, the digestibility of PMS.

(ii) Determine the effect of introducing a second waste stream as a nitrogen source during simultaneous saccharification and fermentation of PMS for hydrogen production.

(iii) Assess the feasibility of using the effluent from hydrogen production for methane production by AD.

(iv) Determine the potential of the resultant digestate for use as a soil amendment.

1.6 Thesis outline

This thesis comprises six chapters, including three experimental chapters, presented in research paper format. Each experimental chapter is independent and contains an abstract, an introduction to highlight the motivation of the study, materials and methods, results and discussion, conclusion, and references.

Chapter 1: An overview that provides background information and the rationale for the study.

Chapter 2: A literature review on paper mill sludge (PMS), its generation, composition and current disposal methods; beneficiation options such as biogas production by anaerobic digestion (AD) and anaerobic co-digestion and production of value-added products; and pretreatment strategies to enhance AD performance. This review is currently being prepared for submission to the Journal of Environmental Management.

Chapter 3: Focuses on modelling and optimising non-ionic surfactant-assisted green liquor dregs (GLD) pretreatment for PMS, using a combined Response Surface Methodology (RSM) design. This work has been published in the International Journal of Hydrogen Energy.

Chapter 4: Assesses the effect of using pharmaceutical wastewater (PW) as a nitrogen source in the modelling and optimisation of dark fermentative hydrogen production from PMS under simultaneous saccharification and fermentation (SSF) conditions. In addition, the kinetics of the optimised processes are assessed using the modified Gompertz model. The work generated in this chapter has been submitted to the journal “Environmental Technology” and is under review.

Chapter 5: Determines the process efficiency of a two-stage AD system using PW as a nitrogen source. The process kinetics are assessed using the modified Gompertz model. Furthermore, the composition of the resultant digestate is assessed for possible use as a soil conditioner. This work has been submitted to the journal “Renewable Energy”.

Chapter 6: This final chapter summarises the thesis, outlining the significance of and integrating the study’s findings. Additionally, conclusions are derived, and recommendations for future work are provided.

1.7 References

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CHAPTER TWO

Literature Review: Beneficiation of paper mill sludge: Current strategies

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2.1 Abstract

The pulp and paper industry generates large quantities of solid waste in the form of paper mill sludge, often disposed of in landfills. Paper mill sludge shows similarity to other lignocellulosic materials but with additional high ash content and trace amounts of heavy metals. Lignocellulosic material is a valuable feedstock for the production of renewable energy and value-added products. This review examines the progress made in paper mill sludge beneficiation and describes sustainable strategies for producing biogas, bioethanol and other value-added products such as lipids and cellulose nanocrystals. Furthermore, techniques to enhance the digestibility of paper mill sludge have been presented. They include anaerobic co-digestion and pretreatment of the sludge to enhance its susceptibility to microbial/enzymatic treatment. Finally, challenges and future prospects of paper mill sludge beneficiation are highlighted.

Keywords: paper mill sludge, beneficiation, biogas, anaerobic digestion, pretreatment

2.2 Introduction

Increasing economic growth and global energy demand have resulted in the implementation of numerous energy policies to increase the production and use of renewable energy, both locally and internationally. This has been made evident by 173 countries implementing renewable energy policy targets and 66 countries with biofuel mandates put in place by early 2016 (Renewables, 2016). Additionally, research conducted on renewable energy generation in the form of solar energy, hydropower, geothermal energy and bioenergy illustrates the peaked interest in developing alternate energy sources (Owusu and Asumadu-Sarkodie, 2016). The renewable nature of energy production from biomass sources is due to biomass energy originating from the sun; however, it can be considered limited as biomass production requires finite resources such as water, arable land, and nutrients (Sánchez et al., 2019). Incorporating organic waste generated from various industries may therefore reduce the competition for biomass utilisation.

Valued at almost 350 billion USD in 2019 (Statista, 2021), the pulp and paper industry is a significant contributor to the global economy. Although the industry uses renewable biomass, its significant water requirement of 50-60 m^3/ton paper (Ram et al., 2020) necessitates water recycling protocols that have led to the generation of solid waste streams that need to be disposed of sustainably. The paper-making process consists of two main phases; conversion of raw fibrous material to pulp and conversion of pulp into paper. Basic operations within these two phases include raw material preparation, pulping (manufacturing, washing, and screening), chemical recovery, bleaching, stock preparation, and paper-making (Bajpai, 2011; Hintz and Lawal, 2018). Processes such as pulping, deinking, and wastewater treatment generate various solid wastes, which are potential pollutants of water, soil, and air (Mandeep et al., 2020). The composition of these wastes is significantly affected by the technologies used during production and wastewater treatment (Bajpai, 2014; Gottumukkala et al., 2016). They include inorganic substances such as ash, green liquor dregs (GLD), lime mud, and slaker grits (Simão et al., 2018), whereas the paper mill sludge (PMS), described as fibrous aggregates suspended in wastewater, makes up the organic portion. Sludge is one of the primary solid organic residues generated from wastewater treatment within the pulp and paper industry, with up to 0.5 million dry tons of sludge generated per year in South Africa (Boshoff et al., 2016) and, due to its complex composition and high water content, is difficult to treat and dispose of. Figure 2.1 illustrates the generation of PMS within the pulp and paper production process.

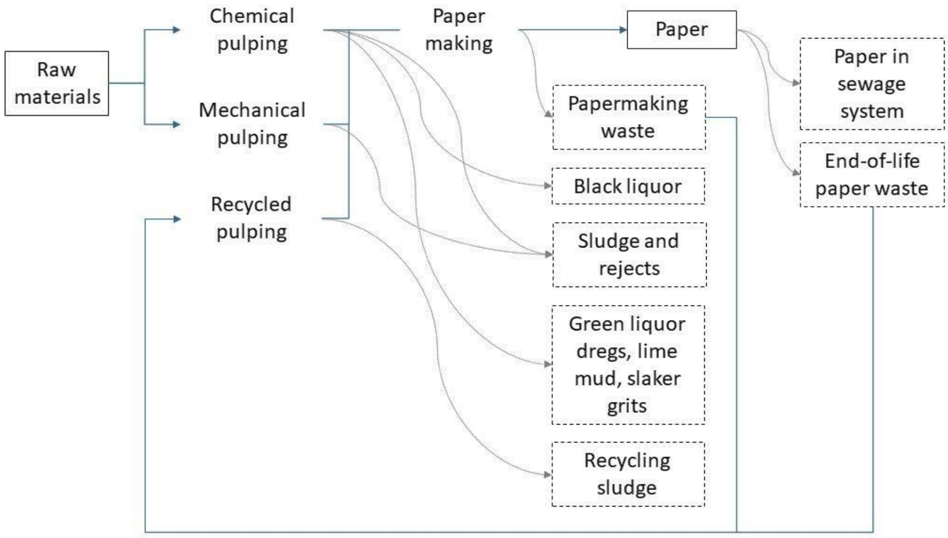

Figure 2.1: Schematic diagram illustrating the process flow of pulp and paper production and PMS production (Adapted from van Ewijk et al., 2018).

Sludge generated by primary clarification during wastewater treatment exhibits high carbon-to-nitrogen ratios (C/N) between 150-250 and generally contains nitrogen, phosphorous, and fillers, but may also contain low amounts of potassium, magnesium, sodium, and toxic metals (Simão et al., 2018). The wastewater from this process is then sent to a secondary biological treatment process which involves the microbiological conversion of soluble organic matter to carbon dioxide (CO_2) and water, resulting in reduced biological oxygen demand (BOD) levels (Simão et al., 2018). Biological sludge (secondary sludge) exhibits a low C/N of 5-30 and is composed of activated sludge, sludge from secondary decanters and thickeners, and cake from the filter press, and typically contains a higher nutrient content than primary sludge (Simão et al., 2018).

The global production of pulp is dominated by the Kraft pulping process, generating approximately 130 million tons of pulp/annum (Shrotri et al., 2017). Carried out with sodium sulphide (Na_2S) and sodium hydroxide (NaOH), the Kraft pulping process is more efficient than sulphite pulping, allowing for faster delignification, shorter cooking times and thus higher pulp integrity (Bajpai, 2018). An addition to the Kraft cooking process known as the prehydrolysis Kraft (PHK) process is a prehydrolysis phase carried out before Kraft cooking. This allows for selective dissolution and removal of wood hemicelluloses (Lahnalammi et al., 2018) before the removal of lignin, generating dissolving pulp (>90% cellulose).

2.3 Current PMS management and disposal techniques

Several management methods are employed for PMS, dominated by landfilling, land application, and energy recovery by thermal processes such as incineration and pyrolysis, all requiring some level of dewatering first. Dewatering is considered a challenging and expensive obstacle in PMS management and has been reported to contribute to more than half the overall cost of wastewater treatment in paper mills, with primary sludge being more dewaterable than waste-activated sludge (Meyer et al., 2018). Dewatering is often achieved by mechanical methods such as sieving, pressing, or centrifugation, requiring high energy demands in the form of electricity. Alternatively, thermal dewatering via evaporation can be employed at a significantly lower cost by drying PMS in well-ventilated rooms or outdoors in the sun. The disadvantage of this, being the large space required (Hagelqvist, 2013a). Therefore, characterisation of the sludge is necessary to determine the most appropriate waste management method. Table 2.1 illustrates the total concentration threshold (TCT) and leachable concentration threshold (LCT) limit range for metal ions in wastes destined for landfills. Should the waste streams exhibit metal ion concentrations exceeding the maximum thresholds, the waste cannot be used in land application and will need to be treated to reduce the concentrations below maximum load restrictions (Herselman, 2013).

Table 2.1: Recommended limits of metal ion concentrations in waste to be disposed of in landfills in South Africa, adapted from Department of Water and Environmental Affairs (2013).

Metal	**TCT threshold range (mg/kg)**	**LCT threshold range (mg/L)**
Arsenic (As)	5.80 - 2000	0.01 – 4.00
Boron (B)	150 - 60000	0.50 - 200
Barium (Ba)	62.50 - 25000	0.70 – 280
Cadmium (Cd)	7.50 - 1040	0.003 – 1.20
Cobalt (Co)	50 - 20000	0.50 – 200
Chromium (Cr)	46000 - 800000	0.10 – 40
Copper (Cu)	16 - 78000	2.00 – 800
Mercury (Hg)	0.93 - 640	0.006 – 2.40
Manganese (Mn)	1000 - 100000	0.50 – 200
Molybdenum (Mo)	40 - 4000	0.07 – 28
Nickel (Ni)	91 - 42400	0.07 – 28
Lead (Pb)	20 - 7600	0.01 – 4.00
Antimony (Sb)	10 - 300	0.02 – 8.00
Selenium (Se)	10 - 200	0.01 – 4.00
Vandium (V)	150 - 10720	0.20 – 80
Zinc (Zn)	240 - 640000	5.00 - 2000

Due to various legislation aimed at banning landfilling, such as the prohibition of landfilling waste with a moisture content above 40% (Department of Environmental Affairs, 2020), the amount of PMS that is landfilled has significantly reduced over the years as industries seek alternative disposal methods (Faubert et al., 2016). In addition to the cost of transportation from paper mills to landfill sites, landfills often require paper mills to pay a gate/tipping fee, significantly increasing the financial burden of sludge disposal.

Land application and composting are suggested as viable options, where the high calcium carbonate ($CaCO_3$) content of PMS has made land application a preferred disposal method in areas where the soil is acidic (Monte et al., 2009). However, PMS often exhibits unfavourable C/N ratios of between 100 and 900 (Camberato et al., 2006; Likon and Trebše, 2012) and is often severely deficient in nitrogen (N) and phosphorous (P) and is therefore inappropriate for use as a soil amendment on its own. Composted PMS is, therefore, mainly utilised to cover discontinued landfills. Deinking sludge and secondary sludge have been reported by Camberato et al. (2006) as low nutrient and high nutrient PMS types, respectively, containing approximately 2.7 gN/kg and 0.1 gP/kg, and 23.3 gN/kg and 4.2 gP/kg, respectively. This considerable variation illustrates the need for comprehensive analyses of each PMS stream prior to subsequent management to ensure the feasibility and success of these techniques and poses a significant financial obstacle to PMS management. Additionally, due to variations in raw materials, the composition of PMS would require regular, comprehensive monitoring, leading to exorbitant costs.

2.4 Energy recovery

Many energy recovery methods have been reported by Monte et al. (2009), including incineration, pyrolysis, steam reforming, and wet oxidation. Incineration of PMS and rejects has become a more commonly employed disposal method as some energy can be recovered in the form of steam. However, due to the high water content of PMS, a 10 °C increase in combustion temperature for every 1% of moisture in PMS means that incineration becomes an energy-intensive method of energy recovery (Likon and Trebše, 2012), resulting in an energy deficient process (Monte et al., 2009). Furthermore, the generation of air emissions, such as sulphur oxides (SOx) and nitrogen oxides (NOx) from incineration, may result in environmental pollution and must be strictly monitored (Shen et al., 2015; Kamali et al., 2019). Nevertheless, one of the main benefits of incineration is reducing the volume of waste, by 80 – 90%, with the final disposal of residual ash in landfills. In addition, the production of steam and/or electricity from thermal energy recovery reduces the dependence of paper mills on the main power grid, with fluidised bed combustion reported as becoming the ultimate final disposal method of paper mill wastes (Monte et al., 2009).

In an effort to create a green economy, more stringent environmental regulations have resulted in industries developing various valorisation techniques to alleviate their environmental footprint. Alternative waste recovery options such as the production of insulation and fire-resistant materials, barrier materials, ceramic and wood composites, and mineral fillers have been reported by Monte et al. (2009), Chimphango et al. (2021) and Barnard (2021). Additionally, to achieve higher total energy recovery, methods that do not require water removal, such as anaerobic digestion, can be employed.

2.4.1 Anaerobic digestion of paper mill sludge

Although up to 19% of PMS is incinerated for energy generation, the economic feasibility of the process is hampered due to the 30-50% water content of PMS (Likon and Trebše, 2012). Anaerobic digestion (AD) of pulp and paper mill wastewater is a well-established treatment process; however, AD of PMS is a relatively new endeavour. Biogas production from organic waste such as PMS shows the potential to significantly contribute to numerous global initiatives to reduce GHG emissions and develop alternative waste management and energy generation techniques (Lindkvist et al., 2019). Bioenergy generation can be in the form of gas, fuel, and steam-derived electricity. During AD, organic matter is degraded by microorganisms in the absence of oxygen as the terminal electron acceptor, generating biogas. The gaseous mixture contains CH_4 (55-75%), CO_2 (25-45%), hydrogen (H_2) (5-10%), and trace amounts of other gases (Kamali et al., 2016). It can be used for heating, upgraded to a higher quality to be used as a natural gas alternative, or used in the co-generation of electricity and heat, with the digestate being recirculated into the soil as a soil amendment (Lindkvist et al., 2019). Methane production by AD occurs via a four-stage, microbiologically-driven process: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Zhang et al., 2016a; Manchala et al., 2017), shown in Figure 2.2. Hydrolytic microorganisms break down complex biopolymers such as carbohydrates, lipids, and proteins into soluble organic compounds such as sugars, long-chain fatty acids (LCFAs), and amino acids. The hydrolytic phase is achieved by extracellular enzymes such as cellulases, proteases, lipases, and amylases secreted by hydrolytic bacteria. The hydrolysis phase is the most time-consuming AD step due to the generation of toxic or unwanted compounds and slow substrate disintegration. Including a pretreatment step before AD can accelerate hydrolysis by aiding substrate disintegration, allowing greater substrate accessibility to anaerobic microorganisms (Náthia-Neves et al., 2018).

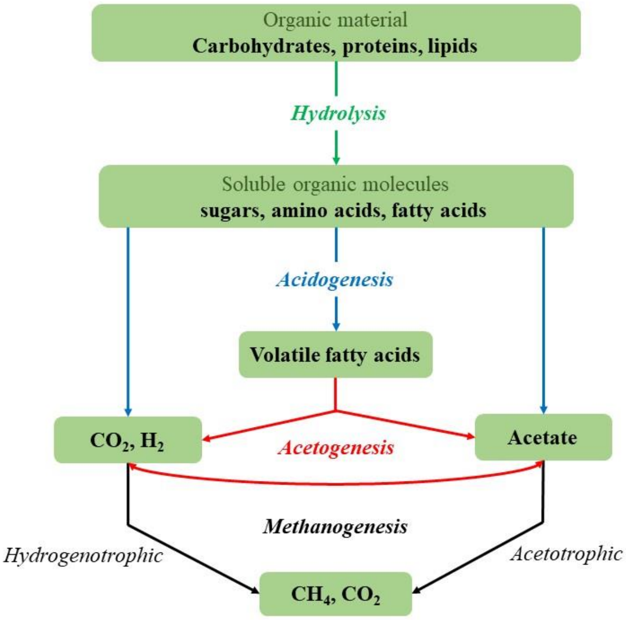

Figure 2.2: Schematic diagram outlining the anaerobic digestion (AD) process.

Soluble organic compounds then serve as both electron donors and acceptors. They are converted to organic acids such as volatile fatty acids (VFAs) (e.g., butyrate, acetate), alcohols, ammonia, hydrogen, hydrogen sulphide, and carbon dioxide by obligate and facultative anaerobic bacteria in the species *Clostridium*, *Propionibacterium*, *Lactobacillus*, etc. The production of organic acids causes a characteristic drop in pH, favouring acidogenic and acetogenic action (Náthia-Neves et al., 2018). Organic acids (VFAs and LCFAs) are converted to precursors for methane formation during acetogenesis, such as acetate, hydrogen, and carbon dioxide. This is achieved by hydrogen-utilising acetogens, which are strictly anaerobic bacteria that use the acetyl-CoA pathway as the primary mechanism for reductive synthesis of acetyl-CoA from carbon dioxide. Organic acids are also oxidised to acetate by hydrogen-producing acetogens. These acetogens are obligate utilisers of hydrogen ions and carbon dioxide as electron acceptors, and are therefore limited by the unfavourable energetics of the process as energy derivation for growth is only possible when product concentration is low. This creates an obligatory dependence on hydrogen scavengers such as sulphate reducers to remove the product. Therefore, this conversion is easily inhibited by high concentrations of hydrogen (> 10^{-4} atm.) in the system. Lastly, all the intermediate products are converted to methane, carbon dioxide, and water by methanogenic microorganisms. This involves acetoclastic/acetotrophic methanogens, which split acetate into methane and carbon dioxide, and hydrogenotrophic methanogens, which use hydrogen as an electron donor and carbon dioxide as an electron acceptor, producing methane in an alkaline environment of 6.5-8.0 (Náthia-Neves et al., 2018).

As an alternative energy recovery method, AD exhibits attractive features, such as;

- The use of wet biomass
- A wide range of suitable substrates
- Low energy requirements at mesophilic temperatures
- Reduced GHG emissions
- Significant retention of fertiliser nutrients such as nitrogen, phosphate, and potassium (K)

The efficiency of the AD process is largely reliant on start-up conditions and environmental factors throughout the process, such as a pH-neutral environment, the absence of oxygen, suitable operating temperatures, among others (Schirmer et al., 2014). Anaerobic digestion can be conducted at psychrophilic (25°C), mesophilic (approx. 35°C), and thermophilic (approx. 55°C) conditions, where microorganisms proliferate best at increased temperatures of mesophilic and thermophilic conditions (Hagos et al., 2017). However, thermophilic AD is more challenging to control since the process is associated with high energy demands and instability when combined with high levels of ammonia (Hagos et al., 2017). In terms of optimum pH, although the variety of microorganisms involved in AD require different pH levels for optimum functionality, the ideal initial pH range of 6.8 – 7.2 has been recommended for AD (Náthia-Neves et al., 2018).

Inhibition of AD can occur due to high concentrations of ammonia, hydrogen, VFAs, and heavy metals. Toxicity and inhibition due to ammonia has been reported to occur in the range of 1500-5000 mg/L, with free ammonia nitrogen (FAN) exhibiting the capability of penetrating cell membranes, leading to proton imbalance, a change in intracellular pH, and inhibition of some enzyme reactions (Akindele and Sartaj, 2018). Inhibition by a build-up of hydrogen occurs in an unbalanced system, where hydrogenotrophic methane production does not occur, which leads to the inhibition of acetogenic action (Anukam et al., 2019). The acidification of an AD system via the accumulation of VFAs causes free acid inhibition due to the protonation of VFAs. Therefore, this inhibition of VFA degradation leads to a further decrease in pH and, thus, further reactor acidification (Franke-Whittle et al., 2014).

Anaerobic digestion studies carried out on PMS are shown in Table 2.2. A low methane yield of 3.5 mL/gVS was obtained in a study by Do Carmo Precci Lopes et al. (2018) from primary sludge, whereas secondary sludge allowed for higher biogas production, generating at least ten times the volume of gas generated by primary sludge. This may be primarily due to the nature of the primary sludge, as inorganic solids and heavy fibrous material are removed in the primary clarifier of wastewater treatment, whereas the clarification of the biological contents of wastewater is removed to form secondary mill sludge (Coimbra et al., 2015). An average yield of 144 mL CH_4/gVS_{added} can be observed from previous studies over 20 – 40 days, illustrating the potential of pulp and paper mill sludge to be used as a resource for methane production. Amare et al. (2019) showed an optimal methane yield of 274 mL/g organic dry matter (oDM) when deinking sludge was processed by AD. The optimal yield was obtained when the C/N of the substrate/inoculum mixture fell between the prescribed ranges of 20-30, demonstrating the significance of C/N when conducting AD. Additionally, although it is reported that high temperatures of thermophilic AD are often preferred due to increased reaction rates (Gebreeyessus and Jenicek, 2016), a review of the literature shows inconclusive results as similarities in process efficiency can be observed in both mesophilic and thermophilic AD.

2.4.2 Co-digestion to improve biogas production

Although AD is a promising method for treating waste, nutritional imbalances in mono-substrate systems result in low yields and high retention times, leading to impractical economics. Anaerobic co-digestion (AcoD) of two or more complementary waste streams has become an economically attractive method to mitigate the low biochemical methane potential (BMP) of AD systems. To ensure process feasibility, chemical composition and characterisation of the substrates must be conducted, and optimisation of operational parameters is essential (Hagos et al., 2017). The suitability of co-digestion substrates is often heavily dependent on the chemical composition of each substrate. Substrates can be classified as carbohydrate-rich, protein-rich, or fat-rich, and combining substrates can balance C/N, pH, solids loading, and nutrient content while diluting inhibitory compounds and increasing microbial diversity (Dima et al., 2020). The effect of C/N on AcoD systems has been studied in depth (Wang et al., 2014; Silvestre et al., 2015; Zahan et al., 2018; Hallaji et al., 2019; Ajayi-Banji et al., 2020; Beniche et al., 2020), illustrating its importance in the success of AcoD systems. Industrial implementation of AD of PMS has been hampered by costly dewatering methods, nutritional deficiency, heavy metal content, and variation in chemical composition (Meyer and Edwards, 2014). Including an additional waste stream that complements the chemical composition of PMS would thus increase its biochemical methane potential, reduce retention times and therefore enhance its economic feasibility. Few studies have focused on the co-digestion of PMS with complementary substrates (Poggi-Varaldo et al., 1997; Hagelqvist, 2013b; Ekstrand et al., 2016; Hagelqvist and Granström, 2016). The co-digestion of PMS with waste sludge and municipal and industrial solid wastes generated between 2.70 and 3.50 L/kg reactor wet mass (rwm).day (Poggi-Varaldo et al., 1997), while high-rate anaerobic co-digestion with activated sludge gave a yield of 220 -240 mL CH_4/gVS (Ekstrand et al., 2016). Haglvqist (2013b) co-digested PMS with municipal sewage sludge and obtained a 58.50% increase in methane yield. These studies demonstrate the enhanced digestibility and methane production potential of PMS when it is co-digested with other waste streams.

Table 2.2: AD studies carried out on PMS

Feedstock	**Process Conditions**	**Inoculum**	**CH_4 Output**	**References**
Pulp and paper mill secondary sludge	55°C, 22% PMS, 20% inoculum conc., pH 6.8, 30 days.	Digestate from a thermophilic municipal wastewater treatment plant, pH 8.1	108 ± 5.0 mL/gVS_{added}	Bayr et al., 2013
Paper sludge	29-32.5°C, 66.6 g/L VS, pH 6.7, 28 days.	None	14.70 mL/gVS	Priadi et al., 2014
Paper sludge	29-32.5°C, 41.28 g/L VS, 20% TS inoculum, pH 6.2-7.3, 28 days.	Cow manure	269 mL/gVS	Priadi et al., 2014
Pulp and paper sludge	37°C, 10% (w/v) PMS, 3.75% (w/v) inoculum conc., 42 days.	Seed sludge from pulp and paper sludge digester, pH 7.85	270 $mL/gVS_{removal}$	Lin et al., 2009
Pulp mill waste activated sludge (WAS)	36°C, 50% inoculum conc., 50% PMS, pH 7.3, 28 days.	Primary sludge from anaerobic digester at a wastewater treatment plant, pH 7.3	404 ± 205 $mL/gVS_{consumed}$	Park et al., 2012
Secondary paper mill sludge	30°C, 10% (w/v) PMS, 10% (v/v) inoculum conc., 33 days.	Digestate from water treatment plant, pH 7.69	173.60 ± 5.87 mL/gVS_{added}	Huiliñir et al., 2014
Activated sludge from pulp and paper mill	37°C, 20% (v/v) inoculum conc., 34-110 days.	Digestate from a wastewater treatment plant	170 mL/gVS	Karlsson et al., 2011
Primary kraft mill sludge	55°C, 2 g VS F/I, 180 rpm, 30 days.	50% UASB enriched with macro and micronutrients, 50% manure	3.50 ± 0.5 mL/gVS	Do Carmo Precci Lopes et al., 2018
Secondary kraft mill sludge	55°C, 2 g VS F/I, 180 rpm, 30 days.	50% UASB enriched with macro- and micronutrients, 50% manure	46.90 ± 9.3 mL/gVS	Do Carmo Precci Lopes et al., 2018

Deinking sludge	37°C, 1.7 S/I (oDM), 21 days	Sewage sludge from a wastewater treatment plant, pH 8.20	274 mL/goDM	Amare et al., 2019
Deinking sludge	37°C, 1.0 S/I (oDM), 21 days	Wastewater AD plant (sewage sludge and food-based residue), pH 8.45	232 mL/goDM	Amare et al., 2019
Deinking sludge	37°C, 0.5 S/I (oDM), 21 days	Digestate from agricultural (maize silage) biogas plant, pH 8.30	217 mL/goDM	Amare et al., 2019
Primary paper sludge	37°C, 10% solids loading, 10% (w/w) inoculum conc., 28 days	Brewery wastewater	49.40 mL/gVS	Williams, 2017
Virgin paper sludge	37°C, 10% solids loading, 30 days	Brewery wastewater	99.50 mL/gTS	Donkor et al., 2021

2.4.3 Pretreatment strategies to enhance amenability of digestion

Due to the complex and resistant structure of lignocellulosic wastes such as PMS, an effort to enhance the economic and practical feasibility of energy recovery by microbial degradation can be made by implementing chemical and/or physical treatment techniques. The effects of pretreatment on lignocellulosic materials include increased accessible surface area and porosity, reduced cellulose crystallinity and particle size, lignin breakdown and removal, and hemicellulose solubilisation (Amin et al., 2017). Naicker et al. (2020) reviewed pretreatment studies that have been conducted, often with the subsequent inclusion of commercial cellulolytic enzymes, such as endoglucanases, β-glucosidases, and cellobiohydrolases. Table 2.3 presents an overview of commonly employed lignocellulosic pretreatment methods. Physical pretreatment includes thermal and mechanical pretreatment and mainly involves size reduction by grinding and milling (Veluchamy and Kalamdhad, 2017a) and is one of the easiest methods to ensure substrate uniformity and increased surface area. However, due to the energy requirement of milling equipment, this process can become laborious and costly. Microwave digestion serves as an alternative physical method, which results in short process times and increased hydrolysis rates. This method is often combined with chemicals to enhance process efficiency (Inan et al., 2015).

A large majority of pretreatment studies have focused on chemical pretreatment. Lignocellulosic material is subjected to acidic or alkaline environments at moderate temperatures and then re-neutralised. Although chemical pretreatment is effective, its industrial application is hampered by high costs and labour-intensive methods and the generation of inhibitory compounds (Veluchamy and Kalamdhad, 2017a). On the other hand, organosolv pretreatment involves organic solvents such as ethanol and acetone for hemicellulose solubilisation and lignin removal. Removal of these solvents before fermentation often increases the cost associated with this pretreatment, which may be offset by the production of high-quality lignin (Badiei et al., 2014). Ozonolysis pretreatment offers a unique route of lignocellulosic disruption by preferred interaction with the electron-rich lignin (Travaini et al., 2016). However, the cost of ozone production significantly reduces the applicability of this method on a large scale. Finally, ionic liquid pretreatment is an environmentally friendly method as there are no toxic chemicals generated from its use. Due to the specific properties of ionic liquids, such as high viscosity, thermal stability and low hydrophobicity, very low energy input is required. Unfortunately, large amounts are required for pretreatment, and the high cost associated with ionic liquids substantially reduces the economic feasibility of this pretreatment (Capolupo and Faraco, 2016).

Table 2.3: Overview of commonly employed pretreatment strategies

Pretreatment	**Effect**	**Advantages**	**Disadvantages**	**Reference**
Mechanical	Cellulose crystallinity disruption Reduced polymerisation Increased surface area of cellulose	Simple process Generation of uniform substrate	Energy-intensive High cost Long process duration	Veluchamy and Kalamdhad, 2017a
Microwave-chemical	Increased cellulose digestibility Increased surface area of cellulose Lignin disruption	Energy efficient No temperature gradient	High energy demand Difficult to scale-up	Inan et al., 2015
Acid	Hemicellulose solubilisation Cellulose reduction Lignin disruption	High glucose release Does not require thermal energy	Production of fermentation inhibitors High cost Severe process conditions	Amin et al., 2017
Alkaline	Lignin removal Increased accessibility to hemicellulose	Mild process conditions Low generation of inhibitors	Production of inhibitory products High cost Production of alkali precipitates Hemicellulose dissolution	Awoyale and Lokhat, 2021
Ionic liquid	Cellulose amorphisation Removal of acetyl groups from hemicellulose Lignin removal	Environmentally benign Recyclable	High cost Long process duration Thermal energy required	Elgharbawy et al., 2016

Organosolv	Cleavage of ether linkages in lignin Lignin dissolution	Reusable Generation of relatively pure lignin	Removal of organic solvent required High cost	Zhang et al., 2016b
Ozonolysis	Lignin depolymerisation Hemicellulose removal	Low inhibitor formation Mild process conditions No chemicals required	Flammable May require subsequent cooling High energy demand	Travaini et al., 2016

The use of commercial enzymes offers the benefit of enhanced solubilisation of lignocellulosic material such as PMS. Dobson (2014) assessed the efficiency of various enzyme cocktails on glucose release during hydrolysis of paper sludge and obtained a maximum glucose concentration of 0.16 g/L from a 2% paper sludge slurry after 48 h hydrolysis using a combination of Novozyme 188 and a CbhII enzyme harvested from *Saccharomyces cerevisiae*. These yields can be significantly increased when enzyme hydrolysis is used in combination with a pretreatment method. Table 2.4 illustrates some of the pretreatment studies carried out on PMS. A major drawback of incorporating enzymatic hydrolysis in the valorisation of PMS is the significant cost of enzymes. This is further exacerbated by high ash contents in PMS, leading to adsorption of lignin and ash on enzymes, which renders the hydrolytic action of enzymes inactive (Naicker et al., 2020). Incorporating an initial pretreatment may therefore reduce the amount required and thus reduce costs. However, due to the vastly different characteristics of sludge generated from different mills, no single regimen can be applied across all types of sludge, and each process needs refinement suited to the traits exhibited by the sludge type. Chemical and/or physical de-ashing may therefore benefit PMS types that exhibit high inorganic fractions, while alkali pretreatments would enhance the digestibility of high-lignin PMS by removing lignin and increasing cellulose accessibility (Kim et al., 2000).

Table 2.4: Pretreatment strategies to improve the digestibility of PMS

Feedstock	**Pretreatment**	**Conditions**	**Improvements**	**Reference**
Pulp and paper mill sludge	Hot air	80°C, 90 min.	25% reduction in cellulose crystallinity	Veluchamy and Kalamdhad, 2017b
Pulp and paper mill sludge	Electrohydrolysis	15V, 45 min., 1:3 solid:liquid	13.80% increase in methane yield	Veluchamy et al., 2018
Recycled paper mill sludge	Ozonation	4 g/h oxygen, 10% sludge, 60-120 min.	43% reduction of soluble lignin, 25% increase in nanocellulose recovery	Peretz et al., 2019
Paperboard mill sludge	Acid catalysed-ionic liquid (choline acetate)	10% IL/PMS + 1% HCl, 120°C, 1 h.	21.37 g/L ethanol yield increase from 0.36 g/L	Farghaly et al., 2017
Kraft paper mill sludge	Sequential aqueous ammonia and hydrogen peroxide	170°C, 30 h.,	10% improvement in digestibility	Kim et al., 2000
Pulp mill waste activated sludge	Alkali/ultrasonic	0.206 g/gTS NaOH, 39.6 MJ/kgTS	80% total methane production reached 5-6 days earlier	Park et al., 2012

2.4.4 Two-stage anaerobic digestion for improved yields

Single-stage AD systems for waste streams are significantly hampered by process instability, VFA accumulation, process inhibition due to ammonia build up and the production of intermediate compounds, reduced handling ability of high organic loads, and low buffering capacity (Srisowmeya et al., 2020). To date, two-stage AD (TSAD) has received increasing interest as it exhibits advantages over a single-stage system, including improved degradation efficiency, increased methane yields, shorter digestion time and higher capacity for increased organic loading rates (Li et al., 2020). By separating the hydrolysis and acidogenic and acetogenic stages, referred to as acidification, from methanogenesis, fermentable sugars that are readily converted to hydrogen and VFAs in acidification are no longer able to inhibit methane production (Pham Van et al., 2019), a schematic illustration of the TSAD process is shown in Figure 2.3.

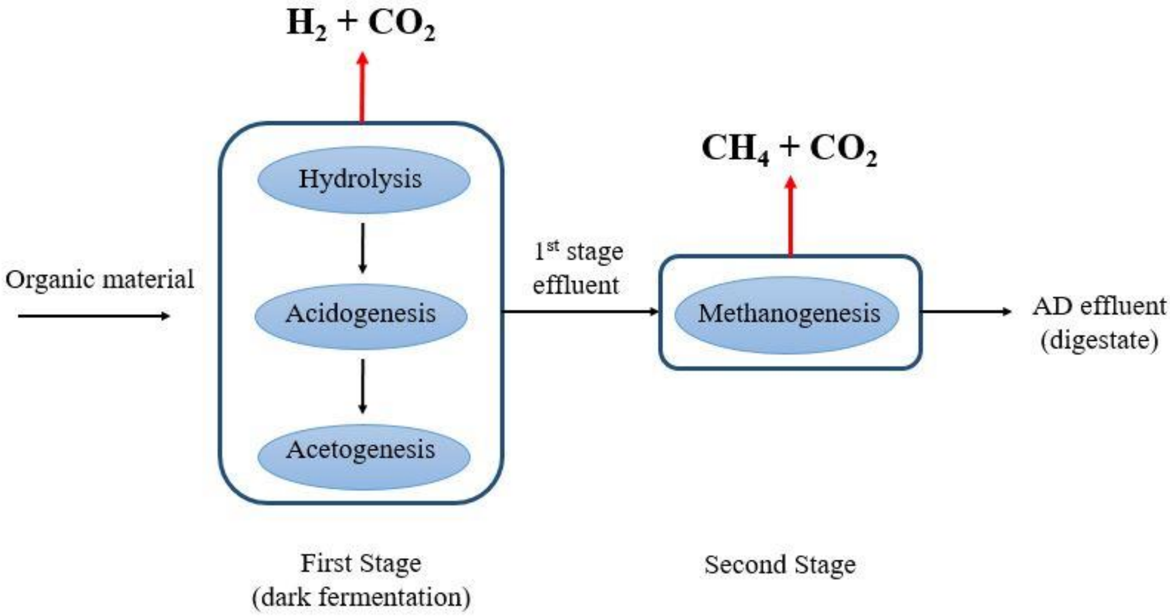

Figure 2.3: Schematic illustration of the two-stage anaerobic digestion process

Furthermore, process conditions required during the two stages differ significantly. For instance, facultative anaerobic microorganisms responsible for hydrolysis and hydrogen production during acidification exhibit optimal growth at a pH range of 5.5 to 6.5. While slower growing, obligate anaerobic methanogenic microorganisms grow optimally at a pH of 6.8 to 7.6 (Nkemka et al., 2015). Providing conditions that are optimal for acidification and methanogenesis, respectively, increases the AD system's performance by increasing the microbial population in each stage by up to five-fold, leading to increased methane production rates (Srisowmeya et al., 2020).

Dark fermentation (DF) is the anaerobic conversion of organic material by acidification to form hydrogen gas, carbon dioxide and VFAs (Bonk et al., 2015). The suppression of methanogenesis is usually achieved by heat treatment of the inoculum or low operational pH (Meyer and Edwards, 2014). The organic acids predominantly formed during acidification are acetate, propionate and butyrate, essential intermediate compounds required for methane production. The longer chained organic acids, such as propionate and butyrate, are often oxidised by hydrogen-producing acetogens, forming hydrogen and additional acetic acid (Annamalai et al., 2020). Although hydrogen exhibits a high calorific value, production by DF often results in low yields and increased VFA generation, rendering the process economically unfeasible. Improving process economics by including pretreatment protocols and directing the effluent of DF to methane production may improve process economics (Cremonez et al., 2021). DF of lignocellulose has been improved with substrate and inoculum pretreatment, resulting in improved hydrolysis rates and, thus, increased fermentable material for hydrogen production. Bundhoo et al. (2015) reviewed inoculum pretreatment and substrate pretreatment strategies implemented for enhanced hydrogen production during DF, reporting heat shock treatment as the most promising inoculum pretreatment for the enrichment of hydrogen-producing microorganisms. Additionally, heat and acid pretreatments were the most commonly employed technologies for substrate pretreatment.

Although literature focused on the effect of C/N in hydrogen production is limited, Srisowmeya et al. (2020) reported a C/N greater than 20 to be optimal for H_2 production stability. This is further substantiated by Lin and Lay (2004), who obtained increased hydrogen yields from sucrose at a C/N of 47. Furthermore, Reyna-Gómez et al. (2019) achieved a maximal hydrogen yield of 86.60 mL/L from fruit peels co-digested with sewage sludge at a C/N of 30. In addition, Wu and Zhou (2012) and Tawalbeh et al. (2021) reported hydrogen by DF of PMS and obtained yields of 620.80 mL H_2/gCOD and 2 mol H_2/mol glucose, respectively. However, there is a lack of comprehensive assessment of DF hydrogen production of PMS in literature.

The accumulation of VFAs during DF is considered one of the main bottlenecks of AD due to the sensitivity of methane producers to VFAs, which leads to a microbial imbalance within the system (Pham Van et al., 2019; Srisowmeya et al., 2020). Therefore, directing the resultant effluent of DF to methane production provides a substrate source rich in VFAs for methanogenesis without the negative impact of VFA accumulation. There are limited accounts of two-stage AD systems for PMS. For example, Lin et al. (2013) reported a maximum yield of 64.48 mL H_2/gVS_{added} and 432.30 mL CH_4/gVS_{added} using PMS co-digested with food waste, achieving a 41.33% VS removal efficiency. In comparison, An et al. (2020) obtained 165 mL H_2/gVS and 376 mL CH_4/gCOD from paper sludge with *Clostridium thermocellum* augmentation during hydrogen production.

Hydrogen and methane produced in each AD stage from biomass can be further combined to form biohythane, comprising 50-60% methane, 30-40% carbon dioxide, and 10-15% hydrogen. Biohythane is a fuel of increasing interest due to its ability to increase automotive combustion efficiency by reducing carbon and nitrous oxide emissions (Cremonez et al., 2021) and has sparked renewed interest in two-stage AD systems.

2.5 The role of modelling and optimisation in process enhancement

Due to the inherent complexity of biological systems, process monitoring, control, and subsequent optimisation is a significant obstacle to achieving process efficiency. One-variable-at-a-time (OVAT) has been the traditional approach to optimisation of fermentation medium formulations. This process involves varying one process input to determine its optimum level while holding all other process inputs constant. The optimum level obtained for the first process input is then applied, and the second process input is determined while holding the remaining inputs constant, usually at the mid-point of the range assessed (Geiger, 2014). The use of OVAT is thus applicable only in situations where interaction between process inputs does not occur. The requirement to conduct numerous experiments makes OVAT a time-consuming and resource-intensive approach that optimises a very small design space. Furthermore, in biological processes, where interactive effects play a significant role in efficiency, true optimisation is not achievable using OVAT.

The introduction of the design of experiments (DoE) concept to optimise chemical and biological processes has gained increasing popularity over the last 20 years. Experimental work is conducted using a systematic approach to eliminate researcher bias, reduce the time required to determine interactive effects of process inputs on product yield and quality, and minimise the cost of process reagents (Weissman and Anderson, 2014). Phases of the DoE process include (Weissman and Anderson, 2014; Das and Dewanjee, 2018; Rajamanickam et al., 2021);

- Planning

The problem of the process is identified, such as low product yield, new product formulation and product quality enhancement. The process response to be modelled and optimised is selected and can include process duration, by-product formation, product purity and product yield. The process inputs most likely to significantly affect the process response are then determined. This information is commonly determined through prior knowledge and historical data obtained through a review of the literature.

- Designing

The number of process inputs and the number of levels of each input that are to be assessed determines the size of the experimental design. The generated design provides the process conditions specific to each experimental run and accounts for replication. Screening process inputs using OVAT and factorial designs to determine whether their variation would significantly affect the process response can also contribute to process design by reducing the design size to focus on the most influential process inputs. These influential process inputs are then selected for process optimisation.

- Optimisation

The experimental design created is then used during optimisation. Each process input is simultaneously varied according to the respective levels within a specified range to obtain optimum process conditions. Response surface methodology (RSM) has gained popularity as an optimisation tool that accounts for interactive effects of process inputs and their role in selected process responses using a polynomial equation. Contour plots generated using RSM graphically illustrate the effect of process inputs on the response.

- Analysis and validation

Analysis of variance (ANOVA) and regression analysis are two of the most common analysis methods applied to process optimisation. Polynomial equations generated by regression analysis provide insight and are assessed based on statistical metrics such as the coefficient of determination (R^2), adjusted R^2 and predicted R^2 values. Statistically sound models which display reasonable root mean squared error (RMSE) values, high R^2, predicted R^2 and adjusted R^2 values are used to predict optimal process responses and experimentally validated to determine their predictive accuracy.

Statistical modelling software such as Design Expert (Stat-Ease, Inc.) and Minitab (Minitab Inc.) allows researchers to develop DoE studies from lab scale to pilot scale, which reduces the pressure on manufacturing and industrial plants to develop optimised processes. This increases the applicability of research conducted in academic institutions to industries, which promotes knowledge transfer. Studies focused on the application of RSM in the enhancement of process efficiency for PMS valorisation are described in Table 2.5.

Table 2.5: Optimisation studies using RSM for enhanced PMS valorisation

Substrate	**Process optimisation**	**Improvement**	**Reference**
Acid (HCl) pretreated primary sludge	Enzymatic hydrolysis for enhanced glucose recovery for ethanol and lipid production	Glucose recovery increased from 1.60% using unwashed primary sludge to 36.90% after pretreatment and 74.4% after optimised surfactant-assisted enzymatic hydrolysis	Zambare and Christopher, 2020
Hydrolysate of enzyme-treated corrugated recycle mill paper sludge	Ethanol production for increased solid loadings and low enzyme dosages	Ethanol production increased from approximately 3.50 g/L using 5-15 FPU/g enzyme dosage to 45.50 g/L using 5 FPU/g	Boshoff et al., 2016
Paper sludge from toilet paper mill	Enzymatic hydrolysis for increased saccharification	Saccharification improved from 45.10% during preliminary screening to 82.10% after optimisation	Peng and Chen, 2011
High ash paper waste sludge	Reactor temperature and pellet size for increased bio-oil yield	Bio-oil yield was improved from *ca.* 25% to 59.90% after optimisation	Ridout et al., 2016
Low ash paper waste sludge	Reactor temperature and pellet size for increased bio-oil yield	Bio-oil yield was improved from *ca.* 20% to 44.50% after optimisation	Ridout et al., 2016
Paper sludge from tissue production	Fed-batch SSF for increased ethanol production	Ethanol concentration was improved from 4.32 g/L to 57.31 g/L after optimisation	Robus et al., 2016

Significant increases in product yield are observed, illustrating the potential of statistical models to enhance process efficiency by reducing enzyme dosages required, increasing the release of fermentable sugar during enzymatic hydrolysis and increasing the quantity of PMS that can be used during fermentation.

In addition to statistical models, kinetic models such as the Monod, modified Gompertz, and Logistic models are commonly used to assess bioprocesses that involve microbial or enzymatic action to improve product quality product yield and process efficiency (Germec et al., 2019). The

Monod model can be used to describe the specific microbial growth rate of a pure culture in a process and relate it to a single, growth-limiting substrate and biomass growth (Kovárová-Kovar and Egli, 1998), while the Logistic model describes microbial growth in a process as a function of growth rate, initial biomass and maximum biomass concentration, and not substrate inhibition (Phukoetphim et al., 2017). In biogas production from complex waste streams such as PMS, a single microbial strain is not commonly employed and instead, a mixed culture serves as the inoculum source. This makes it more difficult to detect and measure microbial growth and substrate degradation, as mixed cultures involve multiple metabolic processes that produce multiple by-products along with the main product. The modified Gompertz model has been compared to the Logistic model to describe the growth of hydrogen-producing bacteria and has, on many occasions, been found to be a more suitable kinetic model (Wang and Yin, 2017). This model describes microbial growth as a function of maximum product concentration, maximum production rate and lag time (Phukoetphim et al., 2017) and can therefore be used in almost all bioprocess modelling studies.

2.6 Value-added products

2.6.1 Ethanol

Ethanol production from various lignocellulosic substrates as a replacement for fossil fuel-derived products has been studied in depth. Additionally, the production of ethanol from PMS has been extensively studied. Robus et al. (2016) reported that at optimal conditions of 20% solids loading and 10-15 FPU/g cellulase loading, a minimum ethanol concentration of 47.72 g/L (0.24 g ethanol/g paper sludge) could be obtained from paper sludge, regardless of its origin. However, the compositional variance observed in paper sludge streams significantly affects product yields. This is illustrated by ethanol concentrations as low as 17.30 g/L obtained by simultaneous saccharification and fermentation (SSF) of paper sludge (Brown et al., 2021). Improvements in process efficiency include studies by Prasetyo et al. (2011), Gurram et al. (2015) and Duncan et al. (2020). Prasetyo et al. (2011) obtained an ethanol yield double that of separate hydrolysis and fermentation (SHF) by implementing simultaneous saccharification and fermentation (SSF), resulting in a yield of 0.23 g ethanol/g organic portion of paper sludge. Gurram et al. (2015) produced 0.49 g ethanol/g glucose using hydrolysate obtained from PMS in the presence of cationic polyelectrolytes. Hydrolysate obtained from enzymatic hydrolysis of PMS was also used by Duncan et al. (2020) to produce up to 0.53 g ethanol/g glucose. Unfortunately, the variation observed in PMS streams causes some difficulty in applying developed methods to various PMS streams. This is illustrated by the ethanol concentrations obtained from Williams (2017), of 27.80 g/L, 39.40 g/L, and 46.80 g/L from tissue printed recycle paper sludge, corrugated recycle paper sludge, and virgin pulp paper sludge, respectively, highlighting the necessity to develop process conditions specific to each PMS stream. Studies dedicated to improving the financial practicality of ethanol production from PMS include Wellington et al. (2021), obtaining an increase in ethanol concentration from 40 to 70 g/L while achieving a 23% savings on the overall enzyme loading required in SSF ethanol production from paper sludge.

2.6.2 Lipids

The microbial production of lipids as feedstock for biodiesel production has gained some interest due to the inexpensive nature of the process. However, very few studies have been reported. For example, Deeba et al. (2016) reported a 15% increase in lipid content (53.40%) and yield (7.80 g/L), using sonicated PMS as a feedstock for triacylglyceride production from *Cryptococcus vishniaccii,* when compared to glucose synthetic medium. While Zambare and Christopher (2020) achieved a lipid content of 37.81% and lipid concentration of 4.46 g/L using surfactant-assisted enzyme-treated PMS as a feedstock for triacylglyceride production from *Cutaneotrichosporon oleaginosum.* This further highlights the potential of PMS as a reliable source of fermentable sugars for various fermentative processes.

2.6.3 Cellulose nanocrystals (CNC)

Due to their specific characteristics, such as high specific strength, thermal stability, and high specific surface area, the rigid, rod-shaped nanofibers which make up CNC can be utilised in several applications, including energy storage, emulsion stabilisation, enzyme immobilisation, as food additives, biosensors, etc. (Trache et al., 2020). For example, Gibril et al. (2018) achieved a 41%, 38%, and 21% CNC yield from prehydrolysis kraft mill sludge, kraft mill sludge, and neutral sulphite semi-chemical mill sludge, respectively, which illustrates that the remaining carbohydrates found in PMS are salvageable for CNC production. In contrast, Peretz et al. (2019) reported a nanocellulose recovery of 0.8% from recycled paper mill sludge, pretreated using ozonation and maleic acid hydrolysis.

2.7 Challenges and prospects

A large amount of waste is generated by the pulp and paper industry globally, with significant amounts of PMS being disposed of, primarily in landfills and some incinerated. Numerous studies have been reported on the treatment of wastewater generated by the pulp and paper industry, with the valorisation of PMS emerging as a practical alternative to the outdated disposal techniques still in use. AD of PMS shows significant potential as an alternative treatment solution; however, several challenges hamper its implementation. One of the major challenges using PMS as the substrate for AD is the nutritional deficit exhibited by most PMS types, which significantly reduces the energy yield and results in extended retention times, leading to an economically unfeasible process. Including a second waste stream that complements PMS characteristics and balances the nutritional value of PMS for AcoD may significantly enhance the feasibility of biogas production from this waste stream. Some challenges associated with this strategy include the requirement of comprehensive characterisation of all waste streams to be used, the implementation of costly pretreatment techniques to enhance the digestibility of PMS, and the disposal of AcoD digestate. Further investigation is therefore required to develop a cost-efficient pretreatment strategy to utilise this waste maximally.

Furthermore, there is little research on how different nutritional sources affect process efficiency regarding the AD of PMS. The high ash content and trace amounts of heavy metals detected in PMS result in a substrate with vastly different characteristics to standard lignocellulosic materials, requiring a tailored approach when used as a substrate for digestion/co-digestion. Nevertheless, PMS shows remarkable potential for microbial conversion. Additional research to develop appropriate strategies for its maximal utilisation will highlight its use as a sustainable, low-cost source of feedstock for the conversion to value-added products. The generation of energy and value-added products from PMS would therefore reduce the environmental risks and costs associated with PMS disposal and, in the case of biogas production; generate a secondary source of energy to reduce the dependence of the pulp and paper industry on the primary power grid, or in the case of value-added products; allow for a secondary revenue stream.

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CHAPTER THREE

Surfactant-assisted green liquor dregs pretreatment to enhance the digestibility of paper mill sludge

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3.1 Abstract

This study optimises a novel surfactant-assisted green liquor dregs (GLD) pretreatment of paper mill sludge (PMS), both of which are wastes from the kraft pulping industry, using a combined Response Surface Methodology (RSM) design. Optimised conditions give a maximal reducing sugar release of 16.38 g/L. A substantial reduction in heavy metals aluminium, chromium, cobalt, arsenic, lead, and copper after pretreatment illustrates the enhancement of substrate digestibility by reducing toxic elements. Separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) for hydrogen production are assessed. SSF produced a hydrogen yield of 3.72 mL/g, displaying a 36.26% increase from pretreated PMS compared to SHF. These findings provide insights into possible methods of reducing process duration, energy input, and costs incurred with waste disposal within the paper industry. Furthermore, improved hydrogen yield using an SSF process demonstrates the potential beneficiation of pulp and paper GLD and PMS wastes.

Keywords: Surfactant-assisted pretreatment, green liquor dregs, lignocellulose, paper mill sludge, SSF hydrogen production.

3.2 Introduction

With the introduction of a green economy, tighter environmental regulations have placed immense pressure on industries to valorise the various waste streams generated. The water and energy-intensive pulp and paper industry has been reported as the fifth largest global energy consumer (Gopal et al., 2019), producing over 400 million tonnes of paper and cardboard each year (Bajpai, 2016; Ouadi et al., 2019). Thus, this industry requires between 300 and 2600 m^3 water per tonne printing and writing paper produced (Gottumukkala et al., 2016) and uses up to 4% of the total global energy supply (Gopal et al., 2019). Therefore, the necessity to implement water recycling protocols has led to waste streams more concentrated in impurities and unrecyclable chemicals. PMS is one of the main waste streams generated by the pulp and paper industry, with up to 40-50 kg dry sludge generated per tonne of paper produced (Bajpai, 2014). The South African pulp and paper industry, in particular, generates up to 500 000 wet tonnes of PMS each year (Boshoff et al., 2016). The cellulose-rich nature of PMS makes this waste a promising source for renewable energy production; however, the presence of impurities, fillers, and clay used during the paper-making process may negatively affect process economics.

Reduced fossil fuel resource availability has also increased energy costs, making it difficult for smaller pulp and paper mills to remain economically viable. Furthermore, landfilling is quickly becoming an unacceptable and costly disposal method, leading to an increased interest in developing low-cost alternative energy production methods (Ouadi et al., 2019) and waste valorisation. The valorisation of PMS would therefore reduce or even eliminate the need for disposal and allow for increased revenue streams by the production of value-added products such as bioethanol, biogas, and biohydrogen, with biogas (50-55.5 MJ/kg) and biohydrogen (141.8 MJ/kg) displaying higher calorific values than bioethanol (29.78 MJ/kg) (Noor et al., 2012; Nwufo et al., 2016). PMS has the additional benefit of reduced lignin content due to the delignification process carried out during paper-making, compared with other lignocellulosic biomass residues such as post-harvest waste, making the PMS less recalcitrant to microbiological processing (Boshoff et al., 2016). Furthermore, using PMS as a feedstock will allow for significant savings regarding transport and disposal costs, while incorporating bioenergy production systems into existing paper mills will significantly reduce infrastructure and energy costs, with the possibility of an additional revenue stream.

Unfortunately, PMS is an industrial waste, and there are several disadvantages associated with its use as a potential renewable energy feedstock. The high water-holding capacity (WHC) of PMS (>0.3 cm^3/g substrate) often leads to a highly viscous fermentation medium, reducing mixing efficiency and mass transfer (Camberato et al., 2006; Villagrá et al., 2011; Boshoff et al., 2016). Furthermore, the high ash content in PMS, particularly that of recycled paper mills, imposes a negative effect on enzymatic hydrolysis due to the irreversible binding of enzymes to ash (Chen et al., 2014), leading to reduced cellulose breakdown and lower product yields. Additionally, the cost-intensive nature of enzyme usage calls for reduced enzyme loading to keep the process economically viable. Attempts to overcome these challenges and thus increase the amenability of PMS to microbial processes by implementing a variety of pretreatment protocols have been reported. Gurram et al. (2015) reported a reduction in ash content of sludge from a kraft pulp and paper mill, from 32.2 to 11% employing chemical de-ashing using hydrochloric acid. This reduction in ash translated to a significant increase in cellulose conversion, from 14.9 to 75.6% after enzymatic hydrolysis. An increase in ethanol production, from 45 g/L to 60 g/L was achieved by Kang et al. (2011) by de-ashing PMS before fed-batch simultaneous saccharification and fermentation (SSF). While Gurram et al. (2015) reported an increase in enzyme hydrolysis performance of 1.8 g/L to 25.9 g/L using 1% hydrogen peroxide pretreatment before enzymatic hydrolysis of de-ashed PMS. Zhu et al. (2019) also reported improved PMS conversion rates, from 45.5 to 56.3 and 55.4% using non-ionic surfactants Triton X-100 (1%) and PEG 8000 (5%), respectively. Therefore, a pretreatment technique suitable to PMS must be developed to obtain the maximum possible fermentable sugar yields while ensuring that the process remains energy and cost-efficient.

Although many pretreatment methods have been developed, the most commonly employed pretreatment regimes use alkali conditions to effectively remove lignin from the remaining cellulose and hemicellulose components of lignocellulosic biomass (Galbe and Wallberg, 2019). The solubilisation and subsequent removal of lignin using alkaline pretreatment occur via ester bond degradation and glycosidic linkage cleavage, which leads to an altered lignin structure, lignin-hemicellulose complex reduction, and partial cellulose decrystallisation (Cheng et al., 2010). Advantages of alkaline pretreatment include low operational costs, reduced holocellulose degradation, and minimized fermentation inhibitor production (Cheng et al., 2010). Residual cellulose and hemicellulose in a solid state are then more amenable to enzymatic attack, resulting in enhanced fermentable sugar production for further value-added product development.

Typically, hydroxides such as NaOH and KOH are used during alkaline pretreatments; however, the formation of salts leads to high costs and low sugar recovery (Martínez-Merino et al., 2013), illustrating the need for cheaper alkali reagents.

Green liquor dregs (GLD) have recently received attention as a cheap and abundantly available alkaline pretreatment agent. GLD is an alkaline waste material generated by sulphate pulp and paper mills during the purification of green liquor. The suspended solids fraction (0.6 – 2.0 g/L) is generally made up of sodium carbonate, calcium carbonate, calcium phosphate, sodium hydroxide, sulphides, unburned carbon, and trace amounts of heavy metals (Golmaei et al., 2017). GLD exhibits a high pH of 11, creating possibilities of its use as an alkaline barrier at landfill sites or as a liming agent. Green liquor has been reported as a pretreatment agent for lignocellulosic biomass (Jin et al., 2010; Saratale et al., 2016; Pham et al., 2018). Additionally, Pöykiö et al. (2006) illustrated the use of GLD as a neutralizing agent for acidic wastewaters; however, GLD is not as commonly used as a pretreatment agent, and there is limited knowledge of its use for PMS.

Furthermore, the use of surfactants such as sodium dodecyl sulphate (SDS), Triton X-100, polyethylene glycol (PEG), and Tween-80 as an enhancement technique during pretreatment has been reported (Pandey and Negi, 2015; Sindhu et al., 2018; Wang et al., 2020). Zhang et al. (2019) reported that non-ionic surfactant Tween-80 enhanced enzymatic hydrolysis by swelling lignocellulosic fibres, increasing surface area, and improving the accessibility of cellulose to cellulase. In a study by Kleingesinds et al. (2018), enhancements can be observed, where the enzyme dosage required to produce high glucose levels was reduced by 41.67% when combined with Tween-80.

This study illustrates the effect of a surfactant-assisted pretreatment protocol using waste GLD to enhance the digestibility and fermentable sugar release from kraft mill sludge by modelling and optimisation for improved fermentable sugar recovery and feedstock digestibility for subsequent bioprocessing and determining effects of the ratio between GLD and PMS, surfactant concentration, solids loading and process duration, using a 3 level and 4 factor combined Response Surface Methodology (RSM) mixture and factorial design.

3.3 Materials and methods

3.3.1 Materials

PMS was acquired from a local Kraft mill in South Africa. Samples were air-dried at 25 °C to a constant weight and stored in airtight containers at room temperature. GLD was obtained from a local paper mill in South Africa. Ionic liquid (BMIM[Cl] (1-butyl-3-methylimidazolium chloride)) was obtained from Sigma-Aldrich, South Africa. Cellulase from *Trichoderma reesei* (Sigma-Aldrich, South Africa) and crude cellulose concentrate (HSL Primafast Gold) (Ecozyme, South Africa (Pty) Ltd) were used for enzymatic hydrolysis. Reagents for buffer and mineral salt solution preparation were purchased from Sigma-Aldrich, South Africa.

3.3.2 PMS screening

To remove contaminants inhibitory to subsequent bioprocessing, such as ash and sand, PMS was screened through a 200 mesh, ten-mesh, and Somerville screen and subsequently oven-dried at 60 °C until constant weight. Resultant PMS fibres were regarded as screened PMS.

3.3.3 Preliminary pretreatment assessment

A preliminary assessment was carried out to determine the feasibility of surfactant-assisted GLD pretreatment of PMS before and after screening. Previously optimised GLD pretreatment conditions (unpublished data) - specifically, a GLD:PMS ratio of 60:40, using a solids loading of 11% (w/v), 1% (v/v) surfactant conc. and a duration of 23 min were used. Additionally, a preliminary assessment of surfactant-assisted ionic liquid pretreatment was carried out, using BMIM[Cl] at 100 °C, for 60 min, or 120 °C for 23 min (Table S3.2). Further, the efficiency of SDS, Tween-80, and Triton X-100 to enhance the effect of GLD pretreatment was assessed (Table S3.3). The resulting PMS was filtered using a domestic sieve (< 1 mm) and neutralized by multiple washings using distilled water. Samples were subsequently oven-dried at 60 °C overnight and stored for enzymatic hydrolysis. The reducing sugar and/or glucose recovery after enzymatic hydrolysis were used as indicators of pretreatment efficiency. Previous GLD optimisation experimentation and preliminary screening determined the significant factors to be optimised using a combined mixture and factorial design. Pretreatment parameter ranges selected for mixture components were GLD concentration (20-80%) and PMS concentration (20-80%) within a solid-to-liquid ratio (S:L) of 5-15% (w/v) corresponded to 1-12% (w/v) for GLD concentration and 1-12 % (w/v) for PMS concentration. Additionally, the effects of varied surfactant (Tween-80) concentration (1-5 % w/v) and pretreatment process time (20-90 min) were assessed. Reducing sugar release was determined using the DNS method (Miller, 1959), while glucose release was determined using Megazyme glucose kits, which measured glucose by colour production of enzymatic reactions of glucose oxidase and peroxidase (Megazyme, Wicklow, Ireland).

3.3.4 Combined mixture and factorial design

Based on preliminary screening data, a combined mixture-process RSM design (Design Expert V12, Stat-Ease Inc., USA) was adopted for the optimisation of significant pretreatment input parameters to determine the interactive effects of the GLD and PMS mixture ratio, surfactant concentration, process duration, and solid-to-liquid ratio (S:L) for maximal fermentable sugar release. Mixture components GLD concentration (20-80% w/v) and PMS concentration (20-80% w/v) as well as factorial input parameters surfactant (Tween-80) concentration (1-5% w/w substrate), process duration (20-90 min) and S:L (5-15% w/v) were considered, which generated a total of 36 experimental runs (Table 3.1). PMS samples were homogenized in distilled water by agitation at 120 rpm for 60 min before pretreatment agents were added. All optimisation pretreatments were carried out at 100 mL working volume in a laboratory autoclave at a temperature of 120 °C. Once pretreated, samples were filtered and neutralized with distilled water as previously described.

Table 3.1: Combined mixture and factorial RSM design for optimisation of surfactant-assisted GLD pretreatment of PMS

Run	**Mixture components**		**Surfactant Conc. (% (w/w))**	**Time (min)**	**S:L (% (w/v))**	**Reducing sugar release (g/L)**
	GLD (% (w/v))	**PMS (% (w/v))**				
1	20.00	80.00	2.76	21.40	10.50	14.20
2	50.98	49.02	4.88	51.50	9.50	13.97
3	50.70	49.30	2.25	88.75	9.50	11.87
4	50.36	49.64	2.25	51.50	14.80	12.01
5	20.00	80.00	1.89	90.00	15.00	11.97
6	80.00	20.00	2.23	51.50	14.85	13.03
7	50.36	49.64	2.25	51.50	14.80	13.57
8	20.00	80.00	5.00	90.00	8.76	13.70
9	80.00	20.00	2.25	88.25	9.50	13.27
10	49.92	50.08	5.00	90.00	15.00	12.70
11	80.00	20.00	3.13	20.00	5.00	12.48
12	20.00	80.00	3.25	65.55	11.50	12.63
13	20.00	80.00	5.00	46.51	15.00	14.63
14	50.00	50.00	5.00	90.00	5.00	14.95
15	50.98	49.02	4.88	51.50	9.50	14.42
16	80.00	20.00	5.00	20.00	15.00	13.59
17	23.00	77.00	0.00	20.00	15.00	12.05
18	80.00	20.00	5.00	90.00	5.00	14.09
19	50.70	49.30	2.25	88.75	9.50	13.37
20	50.32	49.68	0.00	63.40	5.00	12.43
21	23.00	77.00	0.00	90.00	5.00	12.54
22	20.00	80.00	0.00	20.00	5.00	12.43
23	20.00	80.00	2.75	58.50	5.25	12.98
24	48.94	51.06	0.00	90.00	15.00	11.57
25	80.00	20.00	0.00	90.00	15.00	11.17

26	80.00	20.00	5.00	90.00	15.00	13.80
27	49.47	50.53	5.00	20.00	15.00	11.79
28	50.25	49.75	3.13	20.00	5.00	12.81
29	80.00	20.00	0.00	63.91	5.00	10.35
30	20.00	80.00	0.10	58.50	10.50	11.51
31	80.00	20.00	4.90	51.50	9.50	13.87
32	66.50	33.50	0.00	67.95	10.39	11.28
33	23.00	77.00	5.00	20.00	5.00	13.59
34	51.24	48.76	0.00	20.00	11.25	12.14
35	77.60	22.40	2.13	45.20	8.90	12.90
36	80.00	20.00	0.00	20.00	11.15	12.05

3.3.5 Enzymatic hydrolysis of PMS

Surfactant-assisted GLD pretreated biomass was homogenized in 0.05M sodium citrate buffer (pH 4.8) with a working volume of 10 mL by agitation at 120 rpm for one hour at room temperature. An enzyme dosage of 10 FPU/g and solids loading of 5% (w/v) was used at a hydrolysis temperature of 50°C at 120 rpm for 72 h. Biomass samples were then centrifuged at 10 000 rpm for 10 min. The resultant supernatant was then analysed for reducing sugar released after enzymatic hydrolysis using the DNS method (Miller, 1959).

3.3.6 Hydrogen production

Digestate from a lab-based anaerobic digester was used as the source of inoculum in this study. The sludge was heat-treated at 121 °C for 10 min to deactivate the non-spore-forming hydrogen consumers, which are unable to survive at high temperatures while maintaining hydrogen-producing microorganisms (Rorke and Gueguim Kana, 2016). For separate hydrolysis and fermentation (SHF) H_2 production, a substrate concentration of 2.5 gVS was used, with an inoculum concentration of 10% (v/v). A sterile mineral salt solution containing (in g/L): KH_2PO_4 0.5, K_2HPO_4 0.5, $NaHCO_3$ 4.0, $FeCl.2H_2O$ 0.15, $MgCl.6H_2O$ 0.085, $ZnSO_4.7H_2O$ 0.01, $MnCl_2.4H_2O$ 0.03, H_3BO_3 0.03, $CaCl_2.6H_2O$ 0.01, $Na_2MoO_2.2H_2O$ 0.03 (Rorke and Gueguim Kana, 2016) was added at a volume of 50 mL. The initial pH was adjusted to 7 using 5 M NaOH, and the fermentation vessel was flushed with N_2 gas for 30 s, allowing for anaerobic conditions. Fermentation was carried out in a shaking water bath at a temperature of 37.5 °C and 80 rpm agitation for 72 h. Simultaneous saccharification and fermentation (SSF) H_2 production was carried out using a substrate concentration of 2.5 gVS, an inoculum concentration of 10% (v/v), and an initial pH of 6 due to the enzyme activity pH range. Cellulase from *Trichoderma reesei* was used at a dosage of 10 FPU/g in a 0.05 M citrate buffer. A volume of 50 mL sterile mineral salt solution was added, and the fermentation vessel was flushed with N_2 gas for 30 s. Fermentation was carried out at a temperature of 37.5 °C and 80 rpm agitation for 96 h.

3.4 Analytical methods

3.4.1 Compositional analysis

The solubilisation of PMS was analysed using the detergent fibre analysis technique described by Goering and Van Soest (1970) and Wolfrum et al. (2009). PMS was subjected to a neutral detergent solution to solubilize and separate less digestible cell wall components such as cellulose, hemicellulose, and lignin from the digestible cellular components such as starch and sugar. Residual cell wall components were subsequently subjected to an acid detergent solution to solubilize and remove hemicellulose. The remaining components were treated with a $KMnO_4$:lignin buffer solution to solubilize and remove lignin. The final insoluble residue was then ashed to separate cellulose from insoluble ash (Rorke et al., 2017).

3.4.2 Scanning electron microscopy (SEM)

Dried untreated and pretreated PMS samples were visualized using SEM. Samples were placed on adhesive carbon tape and gold sputter-coated using a Quorum 550x sputter coater. Samples were then viewed using a desktop Phenom Pharos scanning electron microscope at 10kV.

3.4.3 Trace elements

Trace elements B, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Mo, Cd, Sn, Sb, Sr, Ba, Pb, and Hg were analysed at the ICP-MS & XRF unit, Central Analytical Facilities at Stellenbosch University, South Africa. An Agilent 7900 quadrupole ICP-MS equipped with a High Matrix Introduction and a 4th generation Octopole Reaction system to remove polyatomic interferences from trace elements of interest. All trace elements were measured in He collision mode, while H_2 reaction gas was used for Se. Elements K, Na, Mg, Si, and P were analysed using a Thermo ICAP 6200 ICP-AES with a charge injection device solid-state detector. Instrument calibration was carried out using a standard series prepared in 2% HNO_3 from NIST-traceable multi-element stock solutions and supra pure, double-distilled acids. ICP-MS standard for Hg was prepared in 2% HNO_3 and 2% HCl. Calibration standards and quality control standards were supplied by Inorganic Ventures (Inorganic Ventures, 300 Technology Drive, Christiansburg, VA 24073) and De Bruyn Spectroscopic Solutions (Bryanston, South Africa). Samples were prepared by microwave digestion (CEM MARS-5). Briefly, between 0.1 and 0.3 g sludge was digested using concentrated supra pure HNO_3 and HCl, cooled, and made up to 50 mL before analysis.

3.4.4 Fourier Transform Infrared (FTIR) analysis

To detect any changes observed in functional groups, sludge samples from each pretreatment step were analysed by FTIR spectroscopy, using a Perkin Elmer 100 (Waltham, MA, USA). Sludge samples were ground using spectroscopic grade KBr and further compressed to produce diameter pellets.

3.4.5 Hydrogen quantification

Biohydrogen volumes were recorded using the water displacement method (Whiteman and Kana, 2014). The hydrogen fraction of the evolving gas was measured using a portable multi-gas analyser (SKZ Industrial, Shandong, China). Cumulative hydrogen volume produced was calculated according to Equation (3.1), where $V_{H,i}$ and $V_{H,i-1}$ are cumulative hydrogen gas volumes at the current (i) and previous (i-1) time intervals, $V_{G,i}$ and $V_{G,i-1}$ the total biogas volumes in the current and previous time intervals, $C_{H,i}$ and $C_{H,i-1}$ the fraction of hydrogen gas in the headspace of the fermentation vessel in the current and previous time intervals, and V_H the total volume of headspace in the fermentation vessel (Prakasham et al., 2011).

$$V_{H,i} = V_{H,i-1} + C_{H,i}\left(V_{G,i} - V_{G,i-1}\right) + V_H\left(C_{H,i} - C_{H,i-1}\right) \qquad (3.1)$$

3.5 Results and Discussions

3.5.1 Preliminary assessment

Initial surfactant-assisted GLD pretreatment screening was carried out in duplicate and used to determine the effect of combined surfactant and GLD pretreatment on releasing fermentable sugar from PMS. Reducing sugar yields of 0.24 g/g PMS were improved to 0.31 g/g PMS, corresponding to a 32% increase in reducing sugar release, from 11.91 to 15.70 g/L, when screened PMS was treated with Tween-80 and GLD. In comparison, pretreatment with GLD alone resulted in a reducing sugar yield of 0.28 g/g PMS, illustrating the enhanced effect of including a surfactant such as Tween-80 to the release of fermentable sugar.

3.5.2 Combined mixture and factorial model development

Experimental conditions and corresponding reducing sugar concentrations obtained are shown in Table 3.1. The experimental data were used to generate a Scheffe cubic x linear model for surfactant-assisted GLD pretreatment. Equation (3.2) relates the concentration of reducing sugar obtained to the various input parameters:

$$Sugar\ release\ \left(\frac{g}{L}\right) = +12.61A + 12.99B - 0.041AB + 1.27AC + 0.052AD + 0.01867AE + 1.43BC - 0.9562BD + 0.0873BE - 2.9ABC + 2.43ABD - 2.59ABE + 33.87AB(A-B) + 47.63ABC(A-B) + 9.91ABD(A-B) + 35.97ABE(A-B) \qquad (3.2)$$

Where A, B, C, D, and E represent PMS (%), GLD (%), surfactant concentration (%), time (min), and S:L (%), respectively.

This model's suitability was assessed using Analysis of Variance (ANOVA), shown in Table 3.2. A coefficient of determination (R^2) of 1 indicates a good fit (Rorke and Kana, 2016). The RSM model generated gave an R^2 value of 0.81, illustrating that the model could account for 81% of the variation observed in experimental data and could relate input parameters to the process response. A high F-value of 5.55, combined with a low p-value of < 0.05 (0.0003), further illustrated the model's significance. Furthermore, each input parameter's suitability was determined by assessing the p-values, wherein input parameter significance was determined by exhibiting a p-value less than 0.05 (Moodley and Gueguim Kana, 2017). Therefore interactions of input parameters AC, BC, BD, ABC exhibited a noted effect on the reducing sugar release for the model.

Table 3.2: Analysis of Variance (ANOVA) of the developed surfactant-assisted GLD pretreatment model

Factor	**Sum of squares**	**df**	**Mean square**	**F-value**	**p-value (prob. > F)**	
Model	32.56	15	2.17	5.55	0.0003	Significant
Linear	0.2684	1	0.2684	0.6858	0.4174	
AB	0.0007	1	0.0007	0.0019	0.9659	
AC	11.17	1	11.17	28.54	< 0.0001	
AD	0.0188	1	0.0188	0.0481	0.8287	
AE	0.2423	1	0.2423	0.6190	0.4406	
BC	8.40	1	8.40	21.45	0.0002	
BD	3.32	1	3.32	8.48	0.0086	
BE	0.0260	1	0.0260	0.0664	0.7993	
ABC	1.94	1	1.94	4.96	0.0376	
ABD	1.51	1	1.51	3.87	0.0633	
ABE	1.68	1	1.68	4.30	0.0514	
AB (A-B)	1.73	1	1.73	4.42	0.0484	
ABC (A-B)	1.77	1	1.77	4.52	0.0461	
ABD (A-B)	0.1063	1	0.1063	0.2716	0.6080	
ABE (A-B)	1.20	1	1.20	3.07	0.0950	
Residual	7.83	20	0.3914	-	-	
Lack of fit	5.39	17	0.3168	0.3890	0.9117	Not significant
Pure error	2.44	3	0.8144	-	-	
Cor total	40.39	35	-	-	-	

A = PMS (%), B = GLD (%), C = Surfactant conc. (%), D = Time (min), E = S:L (%)

3.5.3 Effect of various process parameters on surfactant-assisted GLD pretreatment of PMS

Reducing sugar release values from various experimental runs are shown in Table 3.1, with corresponding response surface plots shown in Figure 3.1.

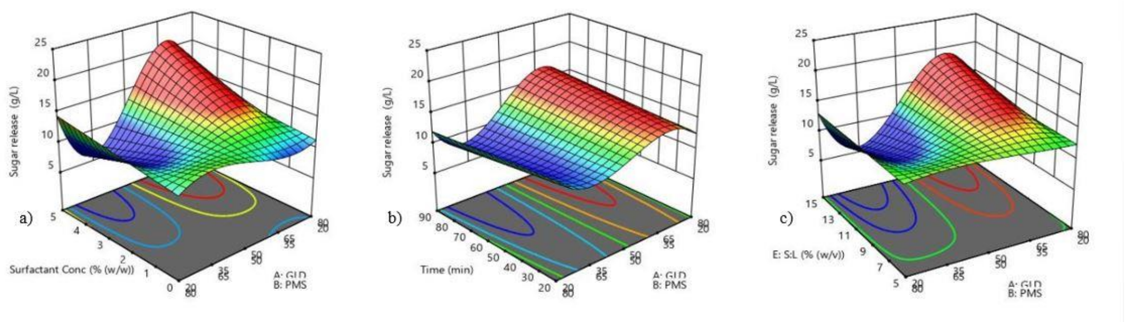

Figure 3.1: 3-D Response surface plots illustrating the effect of various input parameter interactions on reducing sugar release from PMS. PMS to GLD (%) and (a) surfactant (Tween-80) concentration (%), (b) pretreatment time (min) and (c) S:L ratio (%).

The enhanced amenability of PMS to biological degradation, achieved after surfactant-assisted GLD pretreatment, was observed by an increase in the release of reducing sugar, from 10.35 to 14.95 g/L, corresponding to yields of 0.21 to 0.30 g/g. The highest release of reducing sugar was obtained under experimental conditions of 50% GLD, 50% PMS, 5% S:L, 5% Tween-80 conc. and 90 min (run 14); however, a reducing sugar release of 14.63 g/L (0.29 g/g) was obtained when a higher fraction of PMS was used, in combination with a higher S:L of 15% (20% GLD, 80% PMS, 15% S:L, 5% Tween-80 and 46.50 min – run 13). The positive effect of including a surfactant became more significant at higher substrate loadings, thus reducing the time required to obtain a similar yield from pretreatment (Zhang et al., 2019). The interactive effects of GLD to PMS and Tween-80 concentration, while process time and S:L were held at their median point, can be seen in Fig. 3.1a. At low GLD ratios of 20 to 50%, increasing the concentration of Tween-80 had a minimal effect on the release of reducing sugar, with a maximum release of 10 g/L reducing sugar at a Tween-80 concentration of between 1.5 and 2.5%. When the ratio of GLD was further increased from 50 to 80% and PMS was thus reduced from 50 to 20%, an increase in reducing sugar release, from 10 g/L to 15 g/L, was observed. A similar effect has been reported by David et al. (2020), where saturation of GLD in solution resulted in enhanced surface area accessibility, thus providing increased permeation efficiency during enzymatic hydrolysis. At higher ratios of GLD (50-80%), the enhancement of enzymatic hydrolysis by increasing concentrations of Tween-80 (from 2.5 to 5%) was highlighted, increasing the reducing sugar released from 15 g/L to more than 20 g/L. This may be due to the effect of Tween-80 addition, which has been reported to reduce adsorption of cellulase onto lignin and increase desorption of cellulase into the PMS fibres, thus improving the efficiency of enzymatic hydrolysis of pretreated PMS fibres (Zhang et al., 2019). The large increase in reducing sugar release, from <10 g/L (yield of 0.20 g/g) to 22 g/L (0.44 g/g) at an increased concentration of Tween-80 (5%), illustrated the significant impact that the addition of a surfactant had on reducing sugar release after enzymatic hydrolysis of PMS. Furthermore, low PMS concentrations (20-35%) in combination with high surfactant concentration (5%) suggest that the surfactant must be present in saturating quantities, even after pretreatment, to adsorb onto cellulose during enzymatic hydrolysis effectively. Eriksson et al. (2002) reported several ways in which non-ionic surfactants positively affect cellulosic enzyme hydrolysis; a) increasing the surface area of cellulose, b) removing lignin which is inhibitory to enzyme hydrolysis, and c) increasing the stability of enzymes during enzyme hydrolysis.

Fig. 3.1b shows the interactive effects of GLD to PMS and pretreatment time, while Tween-80 concentration and S:L were held at their median points of 2.5% and 10%, respectively. At a low GLD ratio (20%) and high PMS ratio (80%), an increase in pretreatment time, from 20 to 60 minutes, released a reducing sugar concentration of 12 g/L. Increasing the GLD and reducing the PMS concentration ratios from 20:80 to 35:65 resulted in a lower reducing sugar release of 10 g/L – and increasing pretreatment time from 10 to 90 min interestingly led to a broader range of GLD to PMS ratios which produced lower reducing sugar. This negative effect was overcome by further increasing the ratio of GLD to PMS from 35:65 to 65:35 to obtain a maximum of 16 g/L reducing sugar – due to a higher GLD saturation level. The effect of pretreatment time can also be observed to be insignificant at all GLD to PMS ratios, with only a slight increase in its effect at higher GLD to PMS ratios and higher pretreatment times. The interactive effects of GLD to PMS (%) and S:L (%), while Tween-80 concentration and pretreatment time were held at their median points of 2.5% and 55 min, respectively, can be observed in Fig. 3.1c. A low S:L ratio of 5% exhibited a slight effect on reducing sugar release, regardless of the ratio of GLD to PMS implemented. Upon an increase in S:L, from 9 to 15%, a more significant effect of variation in GLD to PMS was observed. Increasing the concentration of PMS (thus reducing the concentration of GLD) displayed a severe negative impact on the release of reducing sugar, resulting in a release of no more than 6 g/L reducing sugar at an elevated S:L ratio of 15% and a GLD to PMS ratio of 35:65. To overcome the negative interaction of a high PMS concentration and high S:L, an increased GLD concentration significantly increased, reducing sugar release to 15 g/L at an S:L of 9% and 19 g/L at an S:L of 15%. The negative impact of increased PMS within the pretreatment system may be due to this substrate's high water holding capacity (Kuokkanen et al., 2008). Steinbach et al. (2017) highlighted the significance of water in the hydrolysis of lignocellulosic biomass, where water at increased temperature and pressure supports cellulose and hemicellulose hydrolysis.

3.5.4 Model validation

Based on model predictions, optimal pretreatment conditions of 56% GLD, 44% PMS, 4.5% Tween-80, 60 min heating time, and S:L of 9.5% should result in a reducing sugar release of 16.70 g/L (0.334 g/g). Experimental validation of the developed surfactant-assisted GLD pretreatment model was carried out in triplicate to determine the model's predictive accuracy, generating an optimal reducing sugar release of 16.38 g/L (0.328 g/g). A 1.92% variation between the predicted and observed reducing sugar release was thus considered negligible.

3.5.6 Structural and compositional impact of surfactant-assisted GLD pretreatment on PMS

Compositional fibre analysis showed that untreated PMS contained cellulose, hemicellulose, and lignin at fractions of 50.13, 7.76, and 40.05%, respectively (Table 3.3). Once screened, the cellulose content increased to 69.96%, while hemicellulose and lignin were reduced to 5.20 and 20.95%, respectively. Subsequent pretreatment displayed a further increase in the cellulose content of PMS to 79.26% and a further decrease in hemicellulose and lignin, to 4.13 and 12.88%, respectively, thus resulting in a total cellulose recovery of 58.11% and a hemicellulose and lignin removal of 46.78 and 67.84% respectively. A study by Yao et al. (2020) reviewed various pretreatment methods, illustrating a 93.9% cellulose recovery and 52.7% lignin removal using 0.6 g H_20_2 and 6 mL green liquor (GL) per gram of dry furfural residues (obtained from corncobs) (Yu et al., 2013) and a 96.5% cellulose recovery and 42.7% lignin removal using a 1:1 ethanol/H_2O and 1 mL GL per gram dry furfural residues (Yu et al., 2014). Additionally, David et al. (2020) reported a cellulose recovery and hemicellulose removal by solubilisation of 42.53 and 47.33%, respectively, using GLD to pretreat corncobs. Similarities observed in the results obtained after GL and GLD pretreatment suggest that this waste exhibits consistency in its ability to mimic strong alkaline pretreatment catalysts, thus effectively cleaving ester bonds between lignin and hemicellulose. Scanning electron micrographs of untreated and pretreated PMS fibres are shown in Figure 3.2. Due to the severe delignification process undertaken during the paper-making process, the PMS generated (Fig. 3.2a) as a waste exhibits morphological attributes similar to alkaline pretreated lignocellulosic biomass (David et al., 2020). The untreated PMS fibre had a smooth and intact surface appearance (Fig. 3.2a) compared with the pretreated PMS fibre surface (Fig. 3.2b), showing a more significant degradation level, where relatively smoother surfaces have become more fibrillated and porous. Thinning and tearing away of the PMS fibre surface seen in Fig. 3.2b highlights the degradative effect of surfactant-assisted GLD pretreatment on PMS and can be attributed to the production of cellulose whiskers (Lima et al., 2014), which leads to increased access to the PMS fibre structure for subsequent microbial degradation.

Table 3.3: Fibre composition of untreated, screened, and pretreated paper mill sludge.

Sample	**Cellulose (%)**	**Cellulose recovery (%)**	**Hemicellulose (%)**	**Hemicellulose removal (%)**	**Lignin (%)**	**Lignin removal (%)**
Untreated	50.13	-	7.76	-	40.05	-
Screened	69.96	39.56	5.20	32.99	20.95	47.69
Pretreated	79.26	58.11	4.13	46.78	12.88	67.84

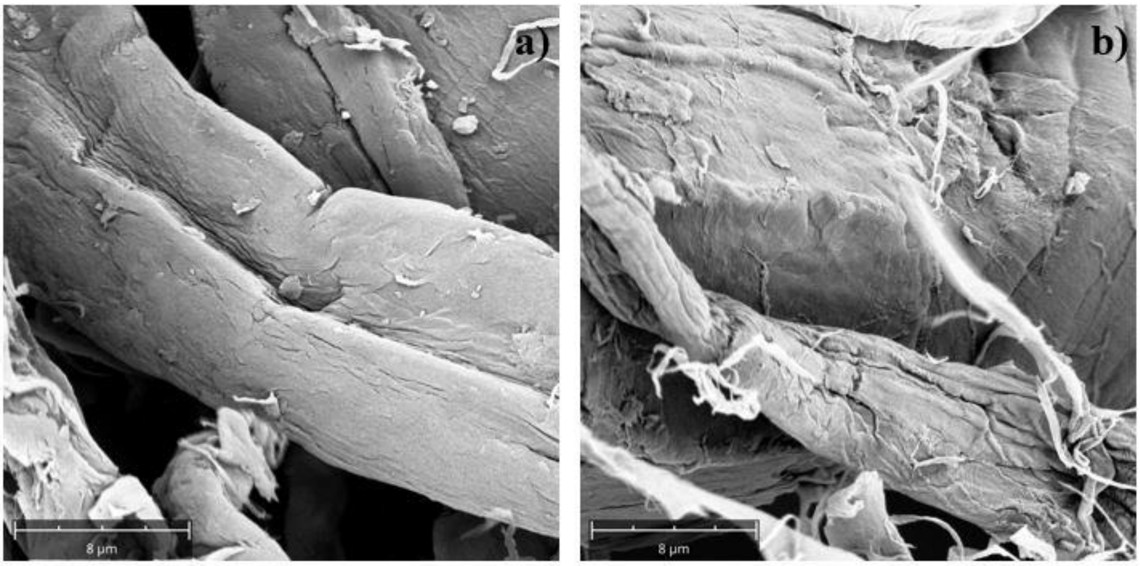

Figure 3.2: SEM images of PMS before and after surfactant-assisted GLD pretreatment. a) untreated PMS and b) optimally pretreated PMS.

FTIR spectra of PMS samples illustrate chemical changes observed after optimal surfactant-assisted GLD pretreatment (Figure 3.3). A reduction in peak intensity is indicative of alterations of functional groups within the PMS (Sodré Souza et al., 2020).

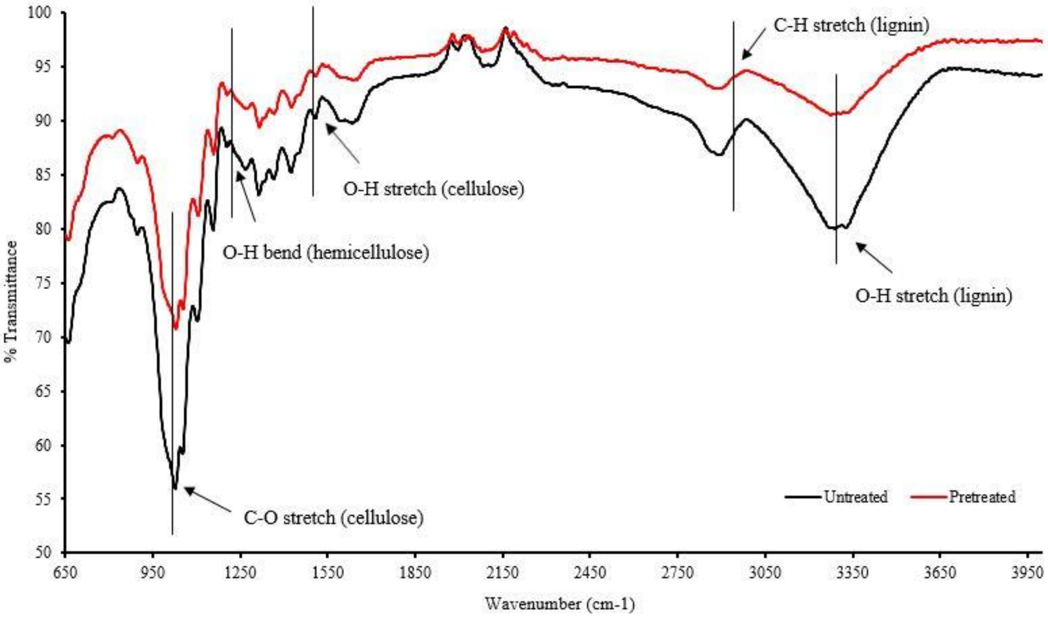

Figure 3.3: FTIR spectra of untreated and pretreated PMS under optimal conditions.

Structural differences between the untreated and pretreated PMS are most evident between 900 and 1100 cm^{-1}, 1250 and 2000 cm^{-1}, 2200 and 3600 cm^{-1}, wherein an increase in percentage transmittance of the pretreated PMS was observed. The untreated and pretreated PMS exhibited pronounced bands at 1028 cm^{-1}, related to a vibration of C-O stretch in cellulose, while band intensity peaks observed at 1506 cm^{-1} were linked to stretching of O-H bonds in cellulose (Sodré Souza et al., 2020). Furthermore, bands observed between 1160 cm^{-1} and 1200 cm^{-1} corresponded with asymmetrical C-O-C stretching and O-H bending in cellulose and hemicellulose (Xu et al., 2013), while bands observed at 900 cm^{-1} linked to stretching of β-glycosidic linkages illustrate the effect of pretreatment on cellulose availability and correlate with the SEM images obtained (David et al., 2020). An increase in percentage transmittance at bands observed at 2907 to 2996 cm^{-1} (C-H stretching in lignin) and 3000 to 3700 cm^{-1} (O-H stretching in lignin) for the pretreated PMS can be attributed to the removal of lignin during pretreatment (Xu et al., 2013; Aruwajoye et al., 2019).

3.5.7 Effect of surfactant-assisted GLD pretreatment on the elemental composition of PMS

ICP analysis was conducted on PMS samples after each step of the pretreatment process to illustrate the effect of each step on the elemental composition of PMS (Table 3.4). Heavy metals are often found in black liquor and thus in wastewater generated by the pulp and paper industry (Aprianti et al., 2018). Detected in quantities below 1 mg/kg were predominantly heavy metals Arsenic (As), Selenium (Se), Cadmium (Cd), Antimony (Sb), and Mercury (Hg). Due to its similarity with phosphate, arsenic is known to be the most toxic heavy metal to humans - found in water, soil, and air (Dhuldhaj and Pandya, 2020; Hare et al., 2020) and has no biological role (non-essential metal). It occurs in four oxidation states, i.e., arsenate and arsenite (inorganic) and elemental arsenic and arsenide (organic) (Dhuldhaj and Pandya, 2020; Hare et al., 2020), with arsenite displaying the most toxicity by binding with sulphur thiolates, forming complexes with glutathione, thus generating free radicals and inhibiting oxidative metabolism by strongly binding pyruvate dehydrogenase (Dhuldhaj and Pandya, 2020). Non-essential metal toxicity in microorganisms has been reported by Dhuldhaj and Pandya (2020) to occur by the displacement of essential metals from their binding sites or by ligand interactions. Microorganisms in particular display varying levels of arsenic resistance, ranging from complete inhibition at 24.70 mg/L (Sharples et al., 1999) to 6000 mg/kg; however, the addition of phosphate has been shown to reduce the toxicity and inhibitory effect of arsenic (Da Costa, 1972). A reduction in arsenic concentration illustrates the removal or reduction of arsenic in PMS, providing a more suitable substrate for subsequent microbial processing.

Table 3.4: Elemental analysis of PMS throughout the surfactant-assisted GLD pretreatment process

Element (mg/kg)	**Untreated**	**Screened**	**Pretreated**	**Enzyme hydrolysed**
Boron (B)	8.64	-	1.01	2.00
Aluminium (Al)	3174.58	1622.70	590.81	501.35
Vanadium (V)	4.18	1.15	0.54	0.48
Chromium (Cr)	28.86	6.46	7.49	5.10
Manganese (Mn)	237.14	64.03	81.88	14.81
Iron (Fe)	1687.20	646.92	293.02	172.90
Cobalt (Co)	2.02	0.57	0.33	0.21

Nickel (Ni)	12.40	2.76	1.48	1.01
Copper (Cu)	31.88	57.07	110.94	34.56
Zinc (Zn)	90.18	66.49	42.39	8.13
Arsenic (As)	0.85	0.14	0.07	0.04
Selenium (Se)	0.07	-	0.06	-
Strontium (Sr)	29.12	15.06	12.00	3.49
Molybdenum (Mo)	1.18	0.35	0.10	0.09
Cadmium (Cd)	0.27	0.11	0.03	0.02
Tin (Sn)	2.04	0.81	0.33	0.55
Antimony (Sb)	0.26	0.10	0.06	0.07
Barium (Ba)	41.72	22.45	12.29	6.13
Mercury (Hg)	0.03	0.04	-	-
Lead (Pb)	8.36	3.65	2.07	1.41
Calcium (Ca)	8672.00	4130.00	3455.90	663.20
Potassium (K)	431.00	92.00	102.90	114.30
Magnesium (Mg)	976.00	661.00	643.00	135.90
Sodium (Na)	3476.00	370.00	287.40	3891.40
Phosphorous (P)	169.00	108.00	27.70	10.90
Silicon (Si)	1154.00	999.00	773.60	842.70

Another non-essential metal detected in untreated PMS was cadmium, at a concentration of 0.27 mg/kg, reduced to a final concentration of 0.02 mg/kg after enzymatic hydrolysis. Megharaj and Naidu (2003) reported an average cadmium content of 0.20 mg/kg in the environment, with elevated cadmium levels in soil due to various anthropogenic activities such as fertilizers, industrial waste, and sewage sludge disposal. Heavy metals such as cadmium have often been reported to be toxic to microbial growth; however, a study by Yu and Fang (2001) illustrated an enhancing effect on the acidogenesis process during anaerobic digestion at cadmium concentrations of less than 20 mg/L. Mercury was detected in untreated PMS at a low concentration of 0.03 mg/kg and increased once the PMS was screened, to 0.04 mg/kg. This increase in mercury may be due to the concentration of the PMS fibres once inorganic components such as clay, fillers, and ash were removed. The toxicity of mercury in microbial communities leads to protein denaturation, cell division and enzyme activity inhibition, nucleic acid destruction, and transcription inhibition (Salam et al., 2019). A minimum concentration of 0.5 µM $HgCl_2$ was reported by Dixit et al. (2004) to be cytocidal, causing cell lysis, while Donkor (2019) reported heavy metal concentrations of 0-4 mg/kg (cadmium) and 0.01-1.4 mg/kg (mercury) in pulp and paper mill sludge, demonstrating the low heavy metal content in the PMS used in this study. A reduction in antimony of 0.19 mg/kg was observed in PMS after enzymatic hydrolysis. Xu et al. (2020) reported similar toxicological and chemical properties to arsenic, with natural antimony concentrations of less than 1 mg/kg found in uncontaminated soil. Li et al. (2016) reported many antimony transformation mechanisms exhibited by microorganisms, including antimony oxidation and dissimilatory antimony reduction. Selenium, detected at low levels in PMS, is readily metabolized by microorganisms; however, the several oxidation states that exist vary in bioavailability, solubility, and toxicity (Staicu et al., 2017), with the oxidized forms of selenium (selenite and selenate) being highly soluble and bioavailable, but toxic (Nancharaiah and Lens, 2015).

Elements detected in the range of 1-5 mg/kg include vanadium, cobalt, molybdenum, and tin. Vanadium in untreated PMS was significantly reduced by 3.70 mg/kg, reaching a final concentration of 0.48 mg/kg after enzyme hydrolysis. Li et al. (2017) reported vanadium as an impurity in petroleum coke, used in kraft pulp mills for lime kilning, at a concentration of up to 1500 ppm. Therefore, vanadium may be due to calcium vanadate formed in the kiln, which then becomes water-soluble $NaVO_3$ when it reacts with Na_2CO_3 in green liquor. Cobalt, a trace metal - detected in the untreated PMS sample at a concentration of 2.02 mg/kg, was reduced to 0.57, 0.33, and 0.21 mg/kg after screening, pretreatment, and enzymatic hydrolysis, respectively. A study by Sairam et al. (2000) assessed the effect of Co^{2+} on H_2 uptake by methane-producing bacteria, resulting in a reduction of H_2 utilization, from 214.5 to 108.4 μmoles/20 mL, illustrating the inhibitory effect of cobalt on microbial processes. Molybdenum, an essential micronutrient that plays a significant role in N assimilation and many other systems requiring enzyme catalysis (Mendel and Bittner, 2006), was present in untreated PMS at 1.18 mg/kg and reduced to 0.09 mg/kg after the enzymatic hydrolysis. Glass et al. (2012) reported a limitation of N_2 fixation at molybdenum concentrations of 1-5 nM in freshwater cyanobacteria. Tin, detected at a concentration of 2.04 mg/kg in untreated PMS, was reported by Cooney and Wuertz (1989) to be present as organo- or inorganotin, both of which show levels of toxicity towards microorganisms. A tin threshold of 750-1000 mg/kg was reported by Lopusiewicz et al. (2019) in a study that tested the influence of tin ions on the development and enzymatic activity of fungal strains; however, there is limited information on the effects of tin on other microorganisms.

Detected at concentrations of 8.64 and 8.36 mg/kg in untreated PMS were boron and lead. Boron was subsequently reduced to 2 mg/kg after enzymatic hydrolysis of PMS. Boron (present in the environment at 0.1-10 mg/L) is a micronutrient essential for N fixation in bacteria but exhibits an inhibitory effect on microbial growth at high concentrations (Miwa and Fujiwara, 2009). A threshold of 3.80 – 47.60 mg/mL of boron compounds such as boric acid and borax was determined by Yilmaz (2012). Lead detected in the untreated sample was reduced to 3.65 mg/kg, illustrating less efficient removal after screening than the reduction observed for boron. Pretreatment reduced the lead concentration by 1.58 mg/kg, with further removal of 0.66 mg/kg after enzyme hydrolysis. Lead is one of the major pollutants, reaching up to 10 000 mg/kg in industrial areas and 200-250 mg/L in industrial wastewater, which is well above the water quality standard of 0.05-0.10 mg/L. Lead content of 1-83 mg/kg was reported by Donkor (2019) in pulp and paper mill sludge. Lead usually binds to clay minerals and complex organic compounds, consequently lowering the lead fraction, which is bioavailable; however, lead is toxic, even at low concentrations (Jaroslawiecka and Piotrowska-Seget, 2014). Despite this, numerous microorganisms have developed mechanisms allowing their survival in environments of high lead concentrations.

Elements detected in the untreated PMS at concentrations between 10 and 30 mg/kg included chromium, strontium, and nickel. Chromium was significantly reduced from 28.86 to 5.10 mg/kg after enzyme hydrolysis. Heavy metals such as chromium are often present in black liquor, which can be recycled into green liquor, from which GLD is obtained (Aprianti et al., 2018). The slight increase in chromium observed in the pretreated PMS sample may be attributed to the possible chromium presence in GLD. Chromium exhibits the most toxicity based on its oxidation state, with Cr(VI) being highly toxic. The reduction of Cr(VI) to lower oxidation states results in free radical production, which causes its toxicity. Chromium levels of 80 mg/kg in paper mill effluents have been reported by Cervantes et al. (2001), which is significantly higher than the observed 28.86 mg/kg in untreated PMS. Strontium, detected at a concentration of 29.12 mg/kg in untreated PMS, is a soft metal, similar to lead, but possesses similar chemistry to that of calcium, readily reacting with halogens, oxygen, and sulphur (Özgür et al., 1996). The strontium concentration was significantly reduced by 25.63 mg/kg after hydrolysis. Due to various uptake mechanisms available for calcium, strontium can accumulate within cells and exhibit toxicity at higher concentrations (Özgür et al., 1996). Nickel was detected in the untreated PMS at a 12.40 mg/kg concentration and was reduced to 1.01 mg/kg after screening, pretreatment, and enzyme hydrolysis. Chromium and nickel concentrations in pulp and paper mill sludge of 1-44 and 1-32 mg/kg, respectively, were reported by Donkor (2019).

Elements detected in the untreated PMS within the range of 30–100 mg/kg were trace metals copper (31.88 mg/kg), zinc (90.18 mg/kg) and barium (41.72 mg/kg). Trace metals are beneficial to maintaining a diverse, healthy microbial community and are often added to wastewater to assist chemical oxygen demand (COD) removal. However, the addition must be applied with caution as high levels of these elements can lead to growth inhibition in various microbial processes (Barnett et al., 2015). The increase in copper observed after screening and pretreatment may be due to copper in distilled water (Cuppett et al., 2006) used in both screening and pretreatment of PMS. Copper was then significantly reduced to 34.56 mg/kg after enzyme hydrolysis and may be linked to a type II copper site in GH61 proteins that play a significant role in enhancing cellulose degradation in *Trichoderma reesei* (Dimarogona et al., 2012). The presence of zinc in untreated PMS was reduced by 82.05 mg/kg after screening, pretreatment, and enzyme hydrolysis, resulting in a final concentration of 8.13 mg/kg. Additionally, barium concentrations were reduced to 6.13 mg/kg after enzyme hydrolysis. The use of barium as a supplemental additive has been shown to increase the activity of extracellular hydrolytic enzymes and may therefore contribute to the hydrolysis of cellulose in PMS (Muñoz et al., 2016).

Elements determined in untreated PMS in the range of 100-300 mg/kg were minerals manganese and phosphorous at concentrations of 237.14 and 169 mg/kg, which were reduced to 14.81 and 10.90 mg/kg, respectively, after enzyme hydrolysis. The minerals potassium and magnesium were reduced to 114.30 and 135.90 mg/kg, respectively, after enzyme hydrolysis. The minerals present in enzymatically hydrolysed PMS can positively impact subsequent microbial fermentation processing of PMS due to their significance in various cellular mechanisms. Elements detected in the range of 1-10 g/kg in untreated PMS were aluminium (3174.58 mg/kg), iron (1687.20 mg/kg), silicon (1154 mg/kg) and calcium (8672 mg/kg) and exhibited reductions of 2673.28, 1514.30, 311.30 and 8008.80 mg/kg respectively after enzyme hydrolysis. Greaves (1922) reported a toxicity of manganese > magnesium > iron > calcium > sodium > potassium, with divalent cations exhibiting higher toxicity than monovalent cations in soil microorganisms. Interestingly, a sodium increase from 287.40 after pretreatment to 3891.40 mg/kg after enzyme hydrolysis was observed, which may be due to the presence of sodium citrate in the citrate buffer used during enzymatic hydrolysis.

3.5.8 Stage-gate analysis of pretreatment protocol for the production of hydrogen

Hydrogen yields obtained after preliminary dark fermentation on PMS samples at each phase of the established surfactant-assisted GLD pretreatment are shown in Table 3.5. It was observed that a 0.15 mL/g (6%) hydrogen yield increase was obtained when untreated PMS was screened and pretreated. A further 9.64% improvement was achieved when screened, and pretreated PMS was enzymatically hydrolysed before hydrogen production. Furthermore, SSF H_2 production on screened and pretreated PMS resulted in a 36.26% yield improvement compared to the hydrogen yield obtained when enzyme hydrolysis was performed in a separate step before hydrogen production. Moreno-Dávila et al. (2017) reported optimal SSF conditions of pH 4, 70 FPU enzyme loading and 45°C, using Celluclast®, obtaining a hydrogen yield of 55 844 mmol/h.gSV from H_2SO_4 pretreated paper industry waste.

Table 3.5: Hydrogen yields obtained after each pretreatment step of the developed surfactant-assisted GLD model.

PMS pretreatment applied	**Hydrogen yield (mL/g)**	**Improvement (%)**
Untreated	-	-
Untreated + EH	2.49	-
Screened	-	-
Screened + EH	2.53	1.61
Screened + SAGLDP	2.64	6.02
Screened + SAGLDP + EH	2.73	9.64
SSF H_2 production		**Improvement (%) ***
Untreated	2.94	18.07
Screened	2.89	14.22
Screened + SAGLDP	3.72	36.26

EH: Enzymatic hydrolysis; SAGLDP: surfactant-assisted GLD pretreatment; SSF: simultaneous saccharification and fermentation; *: Values based on similar pretreatment applied, with EH conducted separately.

These findings illustrate the potential of screened, and optimally GLD pretreated PMS for subsequent use in SSF H_2 production. Including GLD as a pretreatment agent will reduce the energy input and cost required to pretreat lignocellulosic wastes such as PMS while simultaneously providing an alternative use for the waste GLD generated by the pulp and paper industry. Additionally, the use of waste such as GLD as a pretreatment agent for the paper industry's waste PMS would be simple to incorporate into paper mills as the infrastructure required to accommodate these wastes and convert lignocellulose such as PMS at large volumes already exists. Both the pretreatment agent and the lignocellulosic material to be treated originate from the same industry, reducing possible logistical costs. As a result, pulp and paper mills could include biogas production plants within their current infrastructure, with the intention of waste valorisation, which will improve the economics and profitability of renewable energy production.

GLD is a waste that is often landfilled, negatively affecting the pulp and paper industry's economics and the environment in which it is disposed of (David et al., 2020), thus illustrating the potential benefit of redirecting this waste to an avenue of better use.

3.6 Conclusion

This study focused on optimising a novel surfactant-assisted GLD pretreatment and assessed the effect of this pretreatment protocol on the composition and, thus, amenability to microbial processing of PMS. Optimised pretreatment conditions resulted in a reducing sugar release of 16.38 g/L (0.328 g/g). The effect of the pretreatment protocol was assessed by SHF and SSF H_2 production, giving a hydrogen yield of 2.73 and 3.72 mL/g, respectively, illustrating the positive impact of including an enzymatic hydrolysis phase within the production of hydrogen. This study developed an efficient surfactant-assisted GLD pretreatment protocol to enhance the amenability of PMS, which is often regarded as a material that is difficult to degrade or dispose of, using low cost, readily available waste from the same industry as the lignocellulosic material itself. This will ultimately increase the practicality of pretreatment implementation while reducing process costs and energy requirements.

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CHAPTER THREE – APPENDIX

Data shown as supplementary material as per journal specifications are included as supporting information.

Table S3.1: Preliminary assessment of GLD pretreatment of paper mill sludge at 120°C, 23 min.

Pretreatment Conditions	**Reducing sugar/Glucose (g/L)**	
	Unscreened sludge	**Screened sludge**
11% S:L, 60:40 GLD:PMS, 1% SDS	12.48/7.15	10.32/6.39
11% S:L, 60:40 GLD:PMS	9.62/5.49	12.60/6.46
4.4% S:L, 1% SDS	7.99/5.79	12.86/6.62
4.4% S:L	12.34/5.42	11.91/6.06

Preliminary experimentation to determine the effect of GLD pretreatment on paper mill sludge before and after screening showed that the inclusion of a surfactant such as SDS resulted in a reducing sugar release which contained higher glucose content. Unscreened PMS pretreated with GLD and SDS gave a reducing sugar hydrolysate made up of 57.29% glucose, while screened PMS gave a hydrolysate consisting of 61.92% glucose. The lower release of fermentable sugar observed from screened sludge can be explained by the loss of some cellulosic fibre during the screening process.

Table S3.2: Preliminary assessment of surfactant-assisted ionic liquid pretreatment of paper mill sludge at 100°C, 60 min, using BMIM[Cl] and GLD.

Conditions	**Reducing sugar/Glucose (g/L)**	
	Unscreened sludge	**Screened sludge**
BMIM[Cl]		
4.4% S:L, 10:1 IL:PMS, 1% SDS	11.63/6.02	12.23/6.89
4.4% S:L, 10:1 IL: PMS	11.91/5.84	12.10/6.28
4.4 % S:L, 1% SDS	8.59/5.01	13.01/6.50
4.4% S:L	12.67/5.31	11.15/5.94
GLD		
11% S:L, 60:40 GLD:PMS, 1% SDS	-	13.63/7.03
11% S:L, 60:40 GLD:PMS	-	12.05/6.64
4.4% S:L, 1% SDS	8.59/5.01	13.01/6.50
4.4% S:L	12.67/5.31	11.15/5.94
No chemical treatment	12.14/5.83	11.91/6.06

To determine the feasibility of ionic liquid (IL) pretreatment for PMS, a standard ratio of 10:1 was used for IL:PMS. Results obtained were comparable to pretreatment using GLD, which is a significantly cheaper alternative.

Table S3.3: Reducing sugar and glucose recovery observed after surfactant-assisted GLD pretreatment at 120°C for 23 min.

Conditions	**Reducing sugar/glucose release (g/L)**			
	Unscreened sludge	**Screened sludge**		
		Tween 80	**SDS**	**No surfactant**
11% S:L, 60:40 GLD:PMS, 120° C, 23 min	9.62/5.49	15.70/6.79	10.32/6.39	12.60/6.46
11% S:L, 60:40 GLD:PMS, 100°C, 60 min	9.48/6.40	13.83/7.11	13.63/7.03	12.05/6.64
4.4% PMS, 120°C, 23 min	12.34/5.42	14.42/5.95	12.86/6.62	12.01/6.64
4.4% PMS, 100°C, 60 min	12.67/5.31	14.12/8.07	13.01/6.50	11.15/5.94
No additional pretreatment	12.14/5.83	-	-	11.91/6.06

The use of non-ionic surfactants has been reported to enhance enzymatic hydrolysis of lignocellulose significantly. As SDS is an anionic surfactant, its effect during pretreatment was compared to a non-ionic surfactant (Tween-80). Results displayed a marked increase in the reducing sugar release when Tween-80 was used in place of SDS. A 14.77 to 24.60% increase in the reducing sugar concentration was observed when Tween-80 was used during pretreatment, with a more significant improvement observed at 120°C. Therefore, the combination of Tween-80 and GLD was modelled and optimised as a pretreatment protocol for PMS after screening.

CHAPTER FOUR

Effect of pharmaceutical wastewater as nitrogen source on the optimisation of simultaneous saccharification and fermentation hydrogen production from paper mill sludge

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This chapter has been submitted to *Sustainable Chemistry & Pharmacy* with the title: Effect of pharmaceutical wastewater as nitrogen source on the optimisation of simultaneous saccharification and fermentation hydrogen production from paper mill sludge and is currently under review.

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4.1 Abstract

In this study, dark fermentative hydrogen production by simultaneous saccharification and fermentation (SSF) of paper mill sludge (PMS) was developed, using pharmaceutical wastewater (PW) as a nitrogen source to balance the C/N of the system. The effects of cellulase dosage, substrate concentration, pH and nitrogen source were investigated on hydrogen production using response surface methodology (RSM). The process kinetics were evaluated against yeast extract and ammonium nitrate, using the modified Gompertz model. SSF production generated a 2.26% higher hydrogen yield than yeast extract, of 54.79 mL/g PMS, and a peak hydrogen gas fraction of 45.62% when using PW. Optimised processes showed that PW gave comparable kinetic data, exhibited by a 3.26 h variation in process lag time between PW and yeast extract and an 8.73% increase in maximum potential hydrogen production rate when PW was used in place of ammonium nitrate. Implications from this study suggest that using waste to enhance the beneficiation and treatment of waste PMS can sufficiently support fermentation and could significantly reduce the costs associated with its valorisation.

Keywords: simultaneous saccharification and fermentation, dark fermentative hydrogen production, paper mill sludge, response surface model, modified Gompertz

4.2 Introduction

Extensive global dependence on fossil fuel-based energy sources has resulted in environmental pollution, scarcity, and price and market instability (Martins et al., 2019). This has led to an urgency to develop alternative solutions that are cost-efficient, sustainable and safe for the environment. Hydrogen remains one of the most favourable alternative energy carriers, exhibiting an energy content of 122 kJ/g (Rorke and Gueguim Kana, 2016). It is a clean fuel that does not release toxic emissions upon combustion and can be used for electricity generation with ease (Hosseini and Wahid, 2016). However, due to its gaseous state at normal temperature and pressure, hydrogen is more difficult to store and transport (Mori and Hirose, 2009). Numerous hydrogen storage developments have been reported by Hirscher et al. (2020) and Wieliczko and

Stetson (2020), including compression of hydrogen gas using metal hydrides, hydrogen gas liquefaction, and the use of chemical carriers.

Current conventional hydrogen production systems employ hydrocarbon reforming and pyrolysis. Renewable hydrogen production can be carried out by thermomechanical processes such as pyrolysis, gasification, liquefaction and combustion, and biological processes such as bio-photolysis, photo-fermentation, dark fermentation, and sequential dark and photo-fermentation (Nikolaidis and Poullikkas, 2017).

Biological hydrogen production shows great potential as it utilises renewable energy resources such as lignocellulosic material (Das et al., 2008) and functions at ambient temperature and pressure, reducing energy input (Moreno-Dávila et al., 2017). Dark fermentation specifically exhibits advantages over bio-photolysis and photo-fermentation as O_2 is not used during the fermentation, a large variety of organic wastes and effluents can be used as carbon sources, no light illumination is required to maintain constant production, and secondary metabolites such as lactic, butyric and acetic acids can be produced as by-products (Mahidhara et al., 2019). Unfortunately, biological systems currently fall short in practicality due to the lack of standardisation of converting captured solar energy into hydrogen in bio-photolysis and photo-fermentation and low hydrogen yields per unit substrate in dark fermentation (Mahidhara et al., 2019).

The use of abundant lignocellulosic substrates such as post-harvest agricultural waste, paper waste, and woody biomass contributes significantly to the economic viability of biological hydrogen production; however, the recalcitrance of lignocellulose, due to the intricate complex of carbohydrate polymers and lignin, often requires reduction by pretreatment. Physical, chemical, and biological pretreatment techniques are employed to aid in the hydrolysis of lignocellulose, enhancing the accessibility of glucose-containing carbohydrates to enzymatic attack (Moreno-Dávila et al., 2017; Naicker et al., 2020).

In contrast, the wood used in the paper industry undergoes a pulping process where lignin removal significantly reduces the recalcitrance of this material. The pulp and paper industry generates significant quantities of waste as cellulose-rich paper mill sludge (PMS), commonly disposed of in landfills or by incineration. The utilisation of this waste is inundated with low product yields due to high ash content, which results in decreased enzymatic hydrolysis (Tawalbeh et al., 2021). This is often remedied in various ways, such as the inclusion of surfactants which prevents binding between hydrolysing enzymes and ash (Naicker et al., 2020; Rorke et al., 2021) and mechanical de-ashing by fractionation (Chen et al., 2014).

Although PMS is rich in cellulose, it is often deficient in nitrogen (N) and cannot satisfy microbial growth requirements due to regulations that require reduced nitrogen and phosphorus in discharged waste (Slade et al., 2004). For PMS to be usable as a biomass source for energy production, the content of major nutrients and micronutrients needs to be enhanced to ensure that microorganisms that degrade the organic portion of PMS can proliferate. Controlling the carbon-to-nitrogen ratio (C/N) by combining a waste that is low in N with other wastes that are high in N will therefore improve microbial growth and the utilisation of PMS (Farghaly and Tawfik, 2016). Although there is a clear and relatively narrow optimal C/N of 20-30 in literature (Kalil et al., 2008; Wang et al., 2014; Hallaji et al., 2019), very little agreement exists on appropriate nitrogen requirements for hydrogen production. Pérez-Rangel et al. (2020) discussed a high nitrogen requirement, such as C/N ratios in the range of 4-50 for complex substrates such as paper waste hydrolysates and microcrystalline cellulose. This is supported by Lin and Lay (2004), who obtained maximal productivity of 4.80 mol H_2/mol sucrose using a mixed culture at a C/N ratio of 47, and Pérez-Rangel et al. (2020), who reported an optimal C/N ratio of 17.5 using wheat straw as a substrate, producing 504 mL/L.

In contrast, Anzola-Rojas et al. (2015) reported 137 as a suitable C/N ratio for hydrogen production from sucrose-based synthetic wastewater, producing a yield of 3.50 mol H_2/mol sucrose. Wang et al. (2016) employed a mixture of corn steep liquor and corn gluten water as a nitrogen source for enhanced hydrogen production from a co-culture of *Bacillus sp.* A1 and *Brevumdimonas sp.* B1 and obtained a maximal yield of 1.88 mol H_2/mol glucose consumed. This resulted in a 20.8 times increase in the hydrogen production rate than using peptone as a nitrogen source. Furthermore, Kalil et al. (2008) assessed how various N sources impacted hydrogen production using *Clostridium acetobutylicum* and observed an increase in hydrogen yield, from 120 mL H_2/g glucose using ammonium chloride to 240 mL H_2/g glucose using yeast extract. Therefore, employing a waste material high in N would potentially reduce hydrogen production cost while contributing to the optimal production of hydrogen by considering the C/N ratio.

The pharmaceutical industry has become a significant environmental polluter (Kumari and Tripathi, 2019). Effluents from the production of high-quality amino acids and similar pharmaceutical substances show great variability and have been reported by Guo et al. (2017) to exhibit a low C/N ratio. Additionally, wastewater from pharmaceutical industries exhibits high salinity and high levels of nitrogenous compounds, both of which pose a significant challenge to its treatment by negatively affecting microbial activity (Shi et al., 2017). Numerous efforts to reduce the toxicity of PWs by anaerobic treatment have been reported by Shi et al. (2017), from 46.10% COD removal from antibiotic waste (Ng et al., 2015) to rates of up to 99.40% from synthetic brewery waste pharmaceutical industry wastewater (Amin et al., 2006). However, dark fermentative hydrogen production using PW is scantily reported. Hydrogen production of 2.95 mmol/day was obtained by Krishna et al. (2013), using a 1:1 ratio of complex feed and pharmaceutical effluent, while Ghasemian et al. (2019) achieved an average yield of 1.58 mol H_2/mol glucose, using synthetic wastewater. The combination of two recalcitrant wastes for the production of hydrogen is, thus, even less reported.

Dark fermentative hydrogen production from woody materials such as PMS will involve the solubilisation of carbohydrates in PMS by enzyme hydrolysis and subsequent fermentative hydrogen production. To reduce process time, reduce feedback inhibition on cellulosic enzymes by glucose, reduce the number of reactors required and reduce process costs while increasing productivity, the two phases of hydrogen production can be conducted at the same time by simultaneous saccharification and fermentation (SSF). Moreno-Dávila et al. (2017) reported a hydrogen yield of 61.10 mmol/h.gVS using SSF hydrogen production from paper waste, while Giang et al. (2019) reported a hydrogen yield of 170 mL H_2/gVS from biomass of *Chlorella sp.*

This study aimed to enhance the usability of PMS for microbial energy production. Specifically, PW generated from the production of amino acids was assessed against two traditional nitrogen sources: ammonium nitrate and yeast extract, on SSF dark fermentative hydrogen production from PMS, using Response Surface Methodology (RSM). Hydrogen production using each nitrogen source was modelled and optimised using a Box-Behnken design. Optimised hydrogen productivity was then assessed by the modified Gompertz equation.

4.3 Materials and Methods

4.3.1 Microorganisms and materials

The inoculum source was digestate from a lab-based anaerobic digester, with a volatile solids (VS) content of 4.88% (wet wt.). The hydrogen-forming microorganisms in the digestate were heat-inactivated, as described by Rorke et al. (2021). A sterile mineral salt solution containing (in g/L): KH_2PO_4 0.5, K_2HPO_4 0.5, $NaHCO_3$ 4.0, $FeCl.2H_2O$ 0.15, $MgCl.6H_2O$ 0.085, $ZnSO_4.7H_2O$ 0.01, $MnCl_2.4H_2O$ 0.03, H_3BO_3 0.03, $CaCl_2.6H_2O$ 0.01, $Na_2MoO_2.2H_2O$ 0.03 (Rorke and Gueguim Kana, 2016) was used in each hydrogen process. PMS was obtained from a Kraft mill located in South Africa. Samples were air-dried and optimally treated using Tween 80-assisted green liquor dregs (GLD) pretreatment described by Rorke et al. (2021). GLD was acquired from a paper mill in South Africa, whereas Tween-80, cellulase from *Trichoderma reesei*, yeast extract, and ammonium nitrate were procured from Sigma-Aldrich, South Africa. PW, which contained an N content of 1.6%, was obtained from a local amino acid and pharmaceutical production company in South Africa and stored at 4 °C. Trace element analysis on PW and pretreated PMS was conducted at the inductively coupled plasma mass spectrometry (ICP-MS) and X-ray fluorescence (XRF) unit, Central Analytical Facilities at Stellenbosch University, South Africa, following the method described by Rorke et al. (2021).

4.3.2 Preliminary optimisation of C/N ratio

One variable at a time (OVAT) was employed to determine the optimal C/N ratios for hydrogen production, using ratios of 10, 30, and 50 from three different nitrogen sources (Table S4.1). A substrate concentration of 15 g/L was employed, and a volume of 75 mL of a sterile solution of trace minerals was added to the fermentation flask. A 10% (v/v) inoculum concentration was used, along with a pH of 6, adjusted with 5 M NaOH or 1 M HCl. Cellulase from *Trichoderma reesei* was added at an enzyme loading of 10 FPU/g in a 0.05 M citrate buffer. Nitrogen gas was used for flushing the fermentation flask for 60 s, creating an anaerobic environment. Fermentation flasks were incubated in a shaking water bath at 37.5 °C and 80 rpm until gas production ceased. Optimal C/N ratios of 50, 30, and 50 were applied in the subsequent optimisation design for yeast extract, ammonium nitrate, and PW, respectively.

4.3.3 Optimisation of SSF dark fermentative hydrogen production

A Box-Behnken design (BBD) using RSM (Design Expert V12, Stat-Ease Inc., Minneapolis, USA) was adopted to optimise the SSF hydrogen production process, using yeast extract, ammonium nitrate, and PW. Input parameters enzyme dosage (10-150 FPU/g), substrate conc. (5-30 g/L) and pH (5-7) were considered and, with nitrogen source as a categorical factor, a total of 51 experimental runs were generated (Table 4.1). Pretreated and air-dried PMS samples were combined with appropriate quantities of either yeast extract, ammonium nitrate, or PW and inoculated at a concentration of 10% (v/v) of the 150 mL working volume. Adjustment of pH was carried out as described by the experimental design, and cellulase was added. Fermentation vessels were flushed with N_2 gas for 60 s and incubated at 37.5 °C, at 80 rpm. Evolving hydrogen production was recorded using the water displacement method (Rorke et al., 2021), and the hydrogen content of the produced gas was analysed using a portable multi-gas analyser (SKZ

Industrial, Shandong, China). The cumulative hydrogen volume generated was calculated according to the equation described in Rorke et al. (2021) and Prakasham et al. (2011).

Table 4.1: Codes, ranges, and levels of independent parameter variables of enzyme dosage, substrate concentration, pH and nitrogen source in BBD RSM design

Independent variable	**Type**	**Code**	**Low**	**High**
Enzyme dosage (FPU/g)	Numeric	A	10	150
Substrate conc. (g/L)	Numeric	B	5	30
pH	Numeric	C	5	7
Nitrogen source	Categoric	D	Yeast extract, ammonium nitrate, PW	

4.3.4 Assessment of hydrogen productivity using the modified Gompertz model

Data obtained from validated hydrogen production processes were fitted using the modified Gompertz model (Curve Expert V2.7.3, MyBiosource, Inc, USA). This model was used to illustrate the efficiency of each process, as shown in Equation 4.1.

$$H = H_m . \exp\left\{ - \exp\left[\frac{R_m . \exp(1)}{H_m} (t_L - 1) + 1 \right] \right\} \tag{4.1}$$

Where H is cumulative hydrogen production (mL), H_m is maximum potential hydrogen production (mL), R_m is maximum potential hydrogen production rate (mL/h), and t_L is the time from the start of fermentation to exponential hydrogen production (lag time) (h).

4.4 Results and Discussion

4.4.1 Elemental composition of PW and pretreated PMS

ICP analysis was conducted on both PW and pretreated PMS to assess the potential effect of the elemental composition of each material on their respective suitability as substrates for SSF dark fermentative hydrogen production, using general waste discharge standards and drinking water quality standards in South Africa for comparison (Table 4.2). Of the non-essential heavy metals detected, lead (Pb) at a level of 2.070 mg/kg in PMS, and 0.020 mg/L in PW was the highest, exhibiting concentrations much higher than the drinking water quality standard in South Africa (DWQS-SA) of 0.010 mg/L (Verlicchi and Grillini, 2020). Furthermore, a study by Yuan et al. (2015) illustrated the inhibitory effect that increased Pb concentrations had on COD and NH_3-N removal by anaerobic digestion, with a decrease in COD removal efficiency from *ca.* 86% at 1.0 mg/L to 78% at 10.0 mg/L and a decrease in NH_3-N removal from *ca.* 65% at a Pb concentration of 1 mg/L to ca. 30% at 5.0 mg/L.

Arsenic (As), detected at levels of 0.069 mg/kg in PMS and 0.035 mg/L in PW, is considered a highly toxic metal found in soil, water, and air (Hare et al., 2020). A drinking water quality standard of less than 0.010 mg/L (Verlicchi and Grillini, 2020) and a wastewater limit of 0.020 mg/L (Department of Public Works, South Africa, 2012) suggest that the relatively higher As levels may negatively affect microbial communities in anaerobic treatment plants. This may reduce the efficiency of removing toxic components to satisfy regulatory standards. Cadmium (Cd) was detected in PMS at a level of 0.032 mg/kg. It exceeded the general wastewater standard of 0.005 mg/L (Department of Public Works, South Africa, 2012) and the drinking water quality standard in South Africa of no more than 0.003 mg/L (Verlicchi and Grillini, 2020) and has been reported to exhibit high bioaccumulation capacity (Santos et al., 2004).

The study by Santos et al. (2004) found that microorganisms responsible for NH_4-N assimilation were more sensitive to the presence of Cd than the microorganisms responsible for break-down of carbon-rich organic matter, with the presence of Cd in a biological system resulting in reduced sludge quality. Mercury (Hg) was not found in PMS, whereas a very low concentration of 0.001 mg/L was detected in PW, remaining well below the effluent standard of 0.005 mg/L and the drinking water quality standard of 0.006 mg/L (Department of Public Works, South Africa, 2012; Verlicchi and Grillini, 2020).

Table 4.2: Elemental analysis of PW and pretreated PMS before hydrogen production

Element	**Pretreated PMS**[a]	**PW**[b]	**DWQS-SA**[b]	**General waste discharge standards**[b]
Heavy Metals (Non-essential)				
Arsenic (As)	0.069	0.040	0.010	0.020
Cadmium (Cd)	0.032	0.003	0.003	0.005
Lead (Pb)	2.070	0.017	0.010	0.010
Mercury (Hg)	-	0.001	0.006	0.005
Trace elements				
Boron (B)	1.000	3.260	2.400	1.000
Chromium (Cr)	7.490	0.420	0.050	0.050[c]
Cobalt (Co)	0.330	0.030	-	-
Copper (Cu)	110.940	0.190	2.000	0.010
Iron (Fe)	293.020	5.960	0.300-2.000	0.300
Manganese (Mn)	81.880	1.240	0.100-0.400	-
Molybdenum (Mo)	0.100	0.060	-	-
Nickel (Ni)	1.480	0.360	0.070	-
Selenium (Se)	0.060	0.030	-	0.020
Sodium (Na)	287.400	62186	0.200	-
Vanadium (V)	0.540	0.070	-	-
Zinc (Zn)	42.390	1.590	0.005	0.100

Macronutrients				
Calcium (Ca)	3456	79945	-	-
Magnesium (Mg)	643	1797	-	-
Phosphorous (P)	28	39	-	10.000
Potassium (K)	103	3123	-	-
Non-essential elements				
Aluminium (Al)	590.800	2.720	0.300	-
Tin (Sn)	0.330	0.050	-	-
Antimony (Sb)	0.058	0.002	-	-
Barium (Ba)	12.290	3.330	0.700	-
Silicon (Si)	773.600	83.000	-	-
References	This study	This study	Verlicchi and Grillini, 2020	Department of Public Works, South Africa, 2012

a = mg/kg; b = mg/L; c = Cr as Cr(VI)

Trace elements were all detected close to or well above the DWQS and general waste discharge standard in South Africa; however, boron compounds can be tolerated within a range of 3.80 – 47.60 mg/mL (Yilmaz, 2012). The relatively high concentration of chromium (Cr) observed in the PMS may be due to the use of GLD during pretreatment, with Cr levels of up to 80 mg/kg detected in paper mill effluents (Cervantes et al., 2001). Sairam et al. (2000) illustrated the reduction in H_2 uptake by methanogenic bacteria in the presence of Co^{2+}. The low cobalt (Co) levels in both the PMS and PW may enhance hydrogen production by suppressing any remaining methanogenic populations within the digestate used as inoculum. The detection of a high copper (Cu) concentration in PMS may be due to the presence of copper in distilled water used during PMS pretreatment (Rorke et al., 2021). Dimarogona et al. (2012) reported a link between enhanced cellulose degradation during enzyme hydrolysis and a copper site in GH61 proteins. The use of the cellulose-degrading enzyme *Trichoderma reesei* during SSF hydrogen production may therefore be beneficial to the efficiency of the process. Although Iron (Fe) levels exceeded both water and effluent standards, it has been reported that Fe levels of up to 5650 mg/L did not inhibit anaerobic digestion but resulted in reduced biogas production (Jackson-Moss and Duncan, 1990).

Manganese (Mn) detected in PMS and PW at levels of 81.880 mg/kg and 1.240 mg/L exceeded the DWQS of South Africa; however, Qiao et al. (2015) reported an acceleration of methanogenesis by the addition of elemental manganese at a concentration of 4 g/L, illustrating the potential benefit of having Mn in the substrates to be utilised during anaerobic fermentation. Molybdenum (Mo), detected at low levels of 0.100 mg/kg and 0.060 mg/L in PMS and PW, respectively, is an essential micronutrient utilised in microbial nitrogen assimilation found in cofactors of nitrogenase and nitrate reductase enzymes (Glass et al., 2012). The relatively high concentrations of nickel detected in both PMS and PW have been reported by Paulo et al. (2017) to have a possible negative effect on methanogenic cultures at a concentration of 8 mM and should therefore be used with caution. Selenium (Se) detected at relatively low levels in both PMS and PW has been documented to be toxic to the environment and can be converted to a more stable form by anaerobic digestion and then coagulated to remove it from the system (Jain et al., 2016).

A significantly high sodium (Na) concentration (62.190 g/L) was detected in PW and may be due to inorganic chemicals such as sodium hydroxide, sodium bisulfate, and sodium sulfite found in waste generated from chemical waste (Pruss et al., 1999). Vanadium (V) detected at levels of 0.540 mg/kg in PMS, and 0.070 mg/L in PW has been scarcely studied regarding its toxicity towards microbial populations. Efforts to develop a removal system by gravitational settling and sorption onto sawdust from industrial wastewater have been reported by Kaczala et al. (2009), illustrating that both Pb and V sorption can be achieved using wood-based materials. The accumulation of these elements in PMS may therefore be due to this. Zinc (Zn) detected at concentrations above drinking water and effluent standards in both PMS and PW is an essential cofactor utilised by several metalloenzymes when in its ion form (Zn^{2+}); however, at larger concentrations, it becomes toxic to microorganisms (Suryawati, 2018). Macronutrients calcium (Ca), Magnesium (Mg), Potassium (K), and Phosphorous (P) detected in both PMS and PW have the potential to positively impact microbial fermentation as they play significant roles in cellular mechanisms. Aluminium (Al) in PMS at a high level of 590.800 mg/kg has been reported to possess multiple mechanisms of toxicity in microorganisms, including medium acidification, substitution for magnesium, DNA and ATP binding, and the inhibition of ATP synthesis (Piña and Cervantes, 1996).

Tin (Sn) detected in both PMS and PW was observed at significantly lower concentrations than the Sn threshold of 750-1000 mg/kg reported by Lopusiewicz et al. (2019). Antimony (Sb) detected at 0.058 mg/kg, and 0.002 mg/L in PMS and PW, respectively, can be transformed by microorganisms by oxidation and dissimilatory reduction of antimony (Li et al., 2016). The presence of barium (Ba) in PMS and PW can be considered beneficial as the addition of Ba as a supplemental additive has been reported by Muñoz et al. (2016) to increase extracellular hydrolytic enzyme activity and may enhance the production of hydrogen during SSF hydrogen production. Lastly, the elevated presence of silicon (Si) in PMS may result from high silica content observed in some paper mill effluents (Latour et al., 2014). Silica has been reported by Talukder et al. (2017) to inhibit cellulose hydrolysis by adsorbing cellulase, reducing hydrolysis yields by 46%, and should be minimised by the inclusion of polyethene glycol (PEG) 1500 during enzyme hydrolysis of biomass containing silica.

4.4.2 Optimisation of SSF dark fermentative hydrogen production

The experimental data generated using the RSM design gave a reduced cubic model for SSF dark fermentative hydrogen production from PMS. Equations (4.2), (4.3), and (4.4) are final empirical models in terms of actual factors enzyme dosage (A), substrate concentration (B), and pH (C), regarding PW, ammonium nitrate, and yeast extract respectively.

$$H_2\,Yield = +150.31 - 1.23A + 1.20B - 41.40C - 0.001AB + 0.26AC + 0.034BC - 0.001A^2 - 0.09B^2 + 2.14C^2 \qquad (4.2)$$

$$H_2\,Yield = -255.76 + 0.66A + 1.46B + 80.12C + 0.007AB - 0.06AC + 0.53BC - 0.002A^2 - 0.14B^2 - 6.98C^2 \qquad (4.3)$$

$$H_2\,Yield = -24.92 - 0.87A - 3.73B + 41.14C + 0.01AB + 0.16AC + 0.98BC - 0.0005A^2 - 0.08B^2 - 6.10C^2 \qquad (4.4)$$

Model suitability was determined by Analysis of Variance (ANOVA), seen in Table 4.3. RSM model and factor significance are determined by a p-value < 0.05 (Wang et al., 2021). An F-value of 2.86, with a p-value below 0.05 (0.0076) illustrated model significance. Furthermore, an R^2 of 0.7978 indicated that the model accounted for 79.78% of the variation in the data and could relate the input parameters to hydrogen production (Rorke and Kana, 2016). The significance of individual input parameters was determined by assessing p-values < 0.05, and parameter A and parameter interactions AC, BC, and B^2 exhibited a noted effect on hydrogen production.

Response surface plots observed in Figure 4.1 illustrate the interactive effects observed between input parameters. Using PW as an N source, the range in which substrate concentration resulted in higher hydrogen yields increased from ±20 g/L to 12-25 g/L due to the enhancement in hydrolysis observed by increasing enzyme dosage from 10 to 150 FPU/g (Fig. 4.1a). This illustrated that employing a higher enzyme dosage affects the quantity of PMS that can be used during SSF hydrogen production directly, reducing the volume of water required. Furthermore, the combined effect of increasing pH from 5 to 7 and increasing enzyme dosage from 10 to 150 FPU/g seen in Fig. 4.1b increased hydrogen yield, from near 0 to almost 60 mL/gPMS. A comparable finding was reported by Moreño-Davila et al. (2017) when both pretreated and untreated paper industry waste was used for SSF hydrogen production, requiring an increased enzyme dosage for optimal hydrogen production. In contrast, a specific pH of 5 was most beneficial for Moreño-Davila et al. (2017), whereas a maximal pH of 7 resulted in optimal yields in this study, even though the optimal pH for cellulase from *Trichoderma reesei* is pH 6. Figures 4.1c and 4.1d show the interactive effects of enzyme dosage to c) substrate concentration when pH was held at its median point and d) pH, when substrate concentration was held at its median point, using ammonium nitrate as the N source during SSF dark fermentative hydrogen production. A clear optimal substrate concentration range of 15-20 g/L was observed, which was further broadened at increased enzyme dosages, producing a hydrogen yield of up to 40 mL/gPMS.

Table 4.3: Analysis of Variance (ANOVA) of the reduced cubic model developed for SSF dark fermentative hydrogen production

Factor	**Sum of squares**	**df**	**Mean square**	**F-value**	**p-value (prob. > F)**	
Model	12206.35	29	420.91	2.86	0.0076	significant
A- Enzyme dosage	2671.05	1	2671.05	18.14	0.0004	
B- Substrate conc.	63.08	1	63.08	0.4283	0.5199	
C- pH	226.57	1	226.57	1.54	0.2286	
D- N source	471.29	2	235.64	1.60	0.2256	
AB	254.38	1	254.38	1.73	0.2030	
AC	812.14	1	812.14	5.51	0.0287	
AD	60.69	2	30.35	0.2060	0.8154	
BC	719.82	1	719.82	4.89	0.0383	
BD	152.26	2	76.13	0.5169	0.6038	
CD	768.49	2	384.25	2.61	0.0973	
A^2	333.10	1	333.10	2.26	0.1475	
B^2	3356.19	1	3356.19	22.79	0.0001	
C^2	168.23	1	168.23	1.14	0.2973	
ABD	223.64	2	111.82	0.7592	0.4805	
BCD	135.19	2	67.59	0.4589	0.6381	
A^2D	50.94	2	25.47	0.1729	0.8424	
B^2D	197.92	2	98.96	0.6719	0.5214	
C^2D	212.95	2	106.47	0.7229	0.4970	
Residual	3093.01	21	147.29	-	-	
Lack of Fit	1028.75	9	114.31	0.6645	0.7263	not significant
Pure Error	2064.26	12	172.02	-	-	

A= Enzyme dosage (FPU/g), B= Substrate concentration (g/L), C= pH, D= N source as categorical factor.

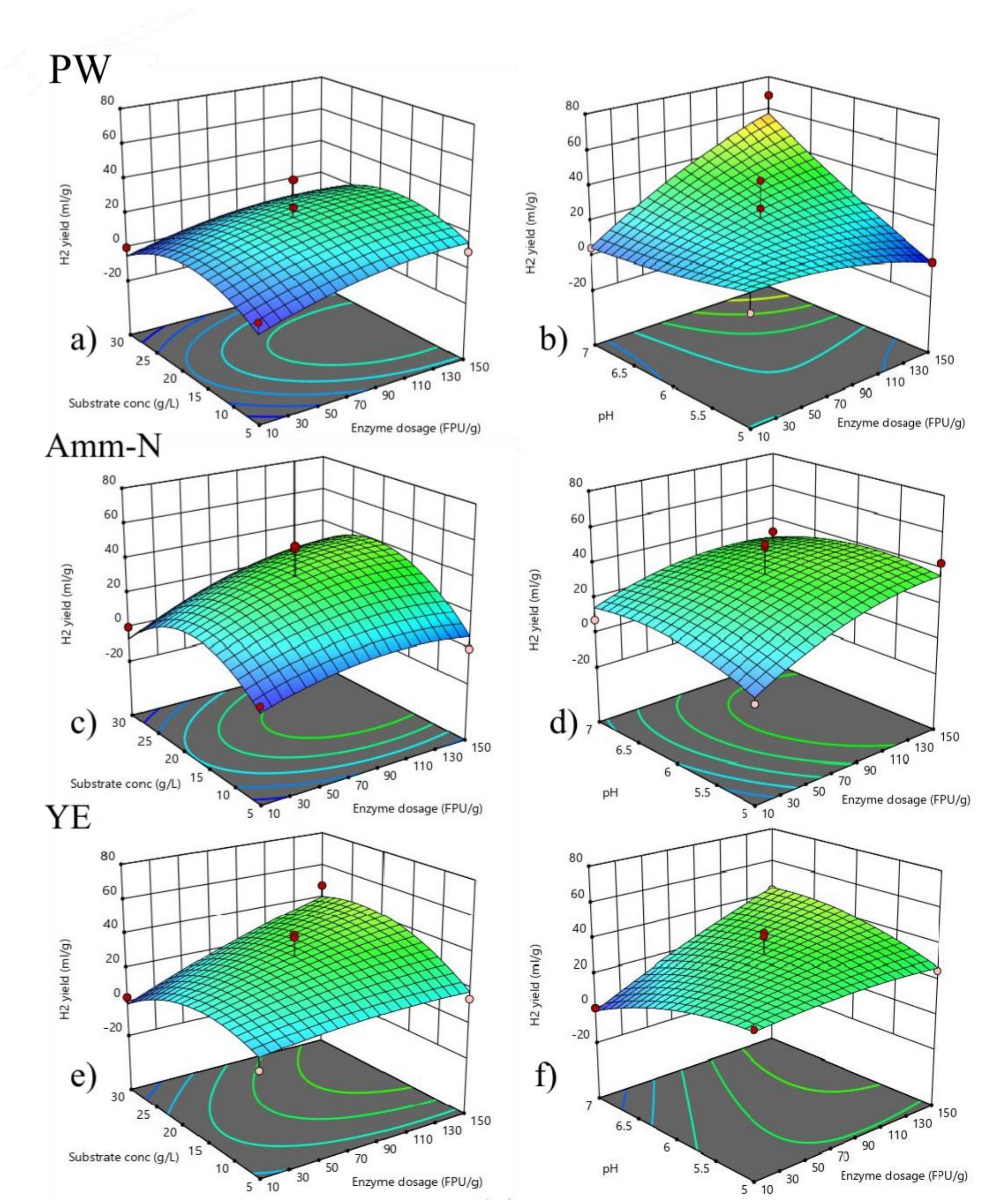

Figure 4.1: Response surface plots displaying the effect of input parameter interactions on hydrogen yield from PMS. Enzyme dosage (FPU/g) and a) substrate concentration (g/L) and b) pH using PW as the N source, c) substrate concentration (g/L) and d) pH using ammonium nitrate as the N source and, e) substrate concentration (g/L) and f) pH using yeast extract as the N source

In contrast to the effect of pH observed using PW as an N source, a lower pH range of 5-6 resulted in higher hydrogen yields (up to 40 mL/g) in the ammonium nitrate supplemented process when a maximal enzyme dosage of 150 FPU/g was employed. This may be due to the inorganic nature of ammonium nitrate serving solely as an N source as it lacks additional growth factors (Clarke, 2013). The interactive effect of substrate concentration and enzyme dosage, when pH was held at its median point using yeast extract as N source, is shown in Fig. 4.1e. A similar broadening of the usable substrate concentration range, from 15-20 g/L to 12-30 g/L at increasing enzyme dosage, was observed, substantiating the enzyme dosage's significance to the yield of hydrogen obtained of up to 50 mL/g. Lastly, the interactive effect of pH and enzyme dosage on hydrogen yield using yeast extract, seen in Fig. 4.1f, shows that the negative impact of increasing pH at low enzyme dosages of 10 FPU/g can be overcome by increasing the dosage to at least 70 FPU/g. This is expected as an increase in enzyme dosage results in increased hydrolysis and an increased concentration of hydrolysed carbohydrates available for microbial fermentation.

4.4.3 Model validation

Model predictions for optimal hydrogen production using PW, ammonium nitrate, and yeast extract are shown in Table 4.4. Experimental validation of the developed model was replicated to determine the predictive accuracy of the model, suggesting an optimal hydrogen yield of 57.62, 40.94, and 55.32 mL/g using PW, ammonium nitrate, and yeast extract, respectively, and resulted in observed yields of 54.79, 39.31 and 53.58 mL/g, corresponding to a range in % error of 3.15-4.88.

Table 4.4: Validation of optimal process conditions predicted for SSF dark fermentative H2 production from PMS

N source	**Enzyme dosage (FPU/g)**	**Substrate conc. (g/L)**	**pH**	**Predicted yield (mL H_2/g PMS)**	**Observed yield (mL H_2/g PMS)**	**% Error**
PW	150.00	18.04	7.00	57.62	54.79	4.88
Amm-N	142.37	20.09	5.87	40.94	39.31	3.98
Yeast extract	150.00	29.18	7.00	55.32	53.58	3.15

4.4.4 Kinetic assessment using the modified Gompertz model

The accumulative hydrogen production and pH evolution of SSF dark fermentation of PMS using PW, ammonium nitrate, and yeast extract are depicted in Figure 4.2. A shorter lag phase was achieved when yeast extract was employed as an N source, with hydrogen production initiated in less than 12 h. This was followed by a lag phase of at least 12 h when PW was utilised, and finally, the initiation of hydrogen production after a duration of 24 h for the ammonium nitrate supplemented process. Hydrogen production rapidly increased from 36 mL to 190 mL between 12 h and 24 h in the yeast extract supplemented process, reaching a peak gas H_2 content of 55.34% at 24 h. A decrease in pH from 7 to 5 observed at 18 h resulted in the reduction in hydrogen volume produced, which led to the deceleration phase observed between 22 h and 26 h. A similar trend in reduced hydrogen production rates due to the pH of the fermentation medium reaching 5, potentially due to the accumulation of hydrogen and volatile fatty acids (VFAs), was reported by Khanal et al. (2004).

A more gradual increase in hydrogen production from 44 mL to 130 mL between 18 h and 42 h was observed in the PW supplemented process, and a peak gas content of 45.62% at 36 h. Again, the initiation of the deceleration phase of hydrogen production observed at 36 h can be associated with the pH of the process reaching 4.9 and thus becoming sub-optimal for hydrogen production, due to a possible shift in the production pathway of microorganisms responsible for hydrogen production (Khanal et al., 2004), from hydrogen and acid production to the production of solvents (solventogenesis) (Penniston and Kana, 2018). The process supplemented with ammonium nitrate; however, displayed a milder increase in hydrogen production, from 77 mL to 100 mL between 36 h and 60 h, and produced a maximum gas content of 37.49% at 42 h, this corresponded with a slight decrease in pH, reaching near 5 at 48 h. Penniston and Kana (2018) reported an extended peak hydrogen production phase under pH-regulated conditions, using glucose as a substrate, with the implication that the onset of solventogenesis and its subsequent reduction in hydrogen production can be mitigated or delayed. Maximal cumulative hydrogen volumes of 202, 132, and 102 mL were obtained, corresponding to hydrogen yields of 46.03, 48.66, and 33.88 mL/g PMS from processes using yeast extract, PW, and ammonium nitrate, respectively.

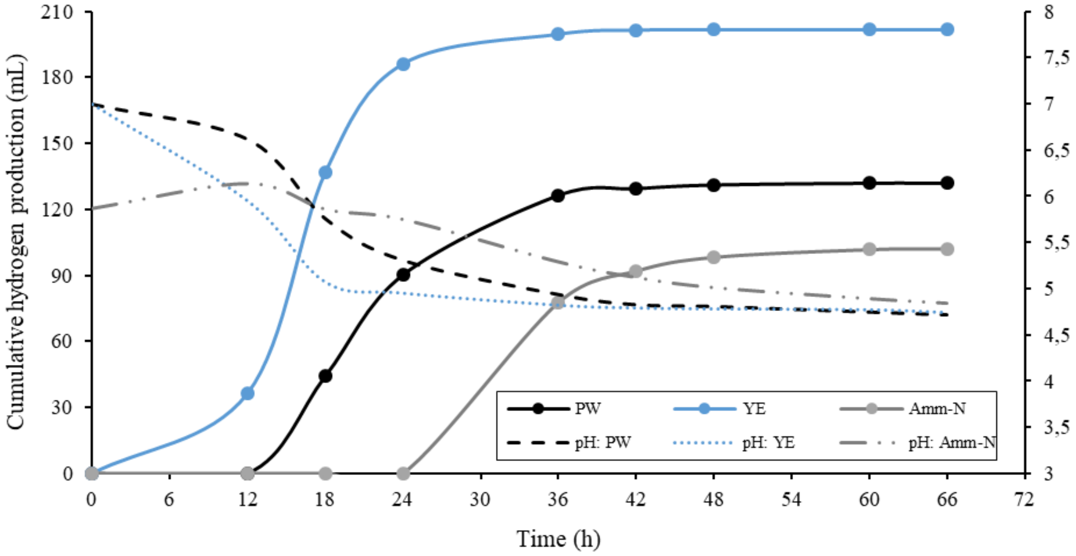

Figure 4.2: Cumulative hydrogen production (mL) and corresponding pH evolution of SSF dark fermentation of PMS using yeast extract (YE), pharmaceutical wastewater (PW), and ammonium nitrate (Amm-N) as N sources

Cumulative hydrogen volumes were fitted using the modified Gompertz model, with high R^2 values for PW (0.99), yeast extract (0.99), and ammonium nitrate (0.99) supplemented SSF processes. A considerable variation in lag times was observed, from 10.17 h for yeast extract to 25.99 h for ammonium nitrate (Table 4.5). A longer lag time may be accounted for by an adjustment phase required by the fermenting microorganisms to the initial pH of 5.87, determined as optimal for SSF hydrogen production using ammonium nitrate. A previous study by Gadhamshetty et al. (2010) reported lag times between 18 and 60 h, using many different hydrogen production systems and glucose or sucrose as a substrate. Therefore, the short lag times observed in this study contribute to reducing energy and costs associated with hydrogen production by dark fermentation, with lower downtime periods. Specifically, PW displayed a lag time of 13.43 h, which was well below reported studies, suggesting that its use as an N source for SSF hydrogen production can be more beneficial to process economics than standard inorganic N sources such as ammonium nitrate. Maximum potential hydrogen production rates for ammonium nitrate, PW, and yeast extract were 8.48, 9.22, and 18.54 mL/h, respectively. Results obtained are consistent with findings by Romão et al. (2014) and Davila-Vazquez et al. (2011), who reported a maximum potential hydrogen production rate of 16.92 mL/h and 19.6 mL/h using a synthetic medium and cheese whey powder, respectively. Yeast extract supplementation of the

SSF process generated a high maximum potential hydrogen production of 201.54 mL, while PW and ammonium nitrate generated 131.41 and 100.89 mL, respectively.

Table 4.5: Hydrogen kinetic parameters achieved for SSF dark fermentation from PMS, using PW, yeast extract, and ammonium nitrate as N sources

Parameter	**N source**		
	PW	**Yeast extract**	**Ammonium nitrate**
H_m (mL)	131.41	201.54	100.89
R_m (mL/h)	9.22	18.54	8.48
t_L (h)	13.43	10.17	25.99

H_m = maximum potential hydrogen production; R_m = maximum potential hydrogen production rate; t_L = lag time.

There is a clear advantage of utilising yeast extract as an N source as it contains peptides, amino acids, vitamins, and trace elements required for microbial growth and metabolism (Zhang et al., 2014), while PW may exhibit some inhibitory effects on hydrogen production due to its high sodium content. A study by Bina et al. (2019) assessed hydrogen production from alkaline wastewater and reported similar maximum potential hydrogen production values of 57.27, 227.51, 208.68, and 97.30 mL using increasing influent alkalinity values of 670, 1325, 2232, and 2678 respectively.

4.5 Conclusion

This study focused on determining the feasibility of utilising PW as an N source for SSF dark fermentative hydrogen production from PMS. Optimised process conditions gave a hydrogen yield of 54.79 mL/g using PW, while yeast extract as an organic N source and ammonium nitrate as an inorganic N source generated hydrogen yields of 53.58 and 39.31 mL/g, respectively. The process kinetics were assessed using the modified Gompertz model, and cumulative maximum hydrogen production values of 131.41, 201.54, and 100.89 mL were determined for PW, yeast extract, and ammonium nitrate supplemented processes, respectively. Thus, this study developed a valuable method of utilising problematic pharmaceutical wastewater, which is low-cost and readily available, to improve the process economics of hydrogen production from waste PMS, which is regarded as a recalcitrant material not easily disposed of. This will enhance the usability of PMS as a substrate for microbial degradation by reducing the time and resources required for successful hydrogen production.

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Competing interests
The authors declare that there are no conflicts of interest.

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CHAPTER FOUR - APPENDIX

Data included as supplementary material as per journal specifications are included as supporting information.

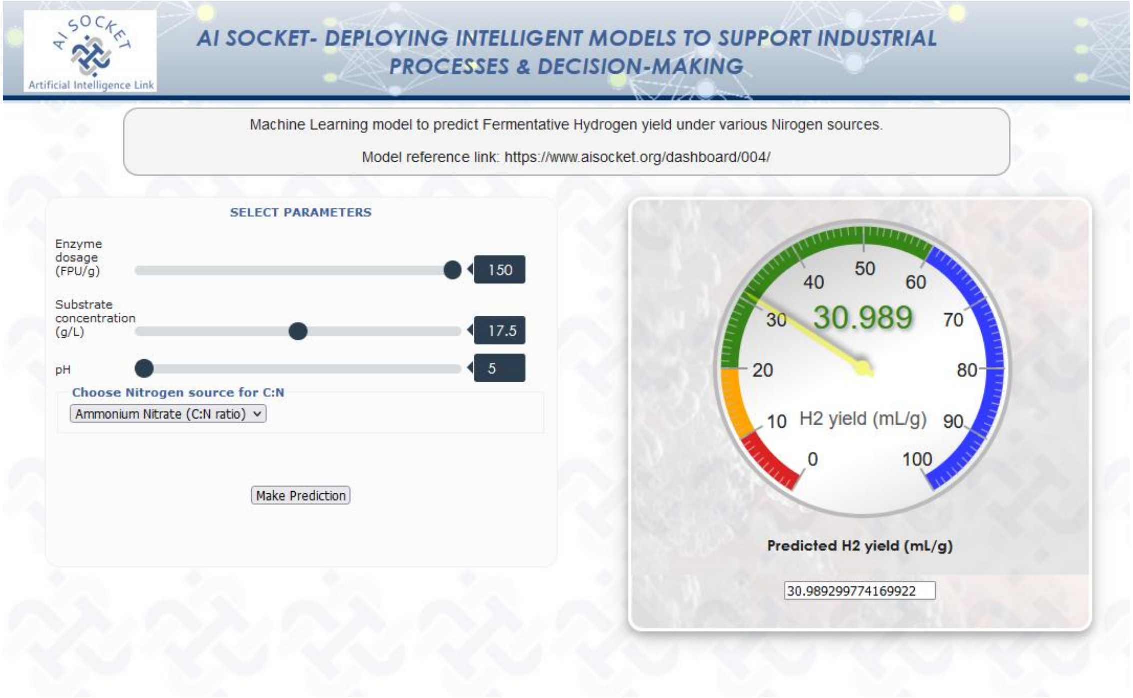
Table S4.1: Cumulative yields observed at varied C/N ratios using yeast extract, ammonium nitrate and PW as nitrogen sources for hydrogen production from PMS

Nitrogen Source	**Cumulative Hydrogen Yield (mL/g)**		
	C/N Ratio		
	10	30	50
Yeast Extract	23.07	13.13	27.25
Ammonium Nitrate	-	6.88	4.49
PW	11.51	13.27	22.52

One variable at a time (OVAT) was used to determine the optimal carbon-to-nitrogen (C/N) ratio for each nitrogen source within the range of 10 – 50. Yeast extract gave a maximal cumulative hydrogen volume of 27.25 mL at a C/N of 50, pharmaceutical wastewater (PW) displayed increasing cumulative hydrogen volumes as the C/N was increased, displaying a maximal volume of 22.52 mL at a C/N of 50. In contrast, ammonium nitrate at a C/N of 30 produced a maximum of 6.88 mL. The optimised C/N ratios obtained were then used in the RSM design.

To promote the use of predictive tools for reduced experimentation, machine learning was used to develop an artificial neural network (ANN) prediction tool using the data generated in this study. Figure S4.1 presents the online dashboard created, which allows the user to select their desired level of each process input. The ANN model then predicts a hydrogen yield using the selected parameters, which can be validated experimentally.

The online dashboard can be found using the link: https://www.aisocket.org/dashboard/004/.



AI SOCKET- DEPLOYING INTELLIGENT MODELS TO SUPPORT INDUSTRIAL PROCESSES & DECISION-MAKING

Machine Learning model to predict Fermentative Hydrogen yield under various Nirogen sources.

Model reference link: https://www.aisocket.org/dashboard/004/

SELECT PARAMETERS

Enzyme dosage (FPU/g): 150

Substrate concentration (g/L): 17.5

pH: 5

Choose Nitrogen source for C:N: Ammonium Nitrate (C:N ratio)

Predicted H2 yield (mL/g): 30.989299774169922

Figure S4.1: Screenshot of the interactive online dashboard of ANN predictive model for SSF hydrogen production from PMS, using PW, yeast extract or ammonium nitrate as the preferred nitrogen source

To improve model prediction accuracy, machine learning requires large datasets. With the increase in research into bio-product formation, extensive literature can be used to develop ANN-derived prediction tools and enhance their predictive accuracy. Using a limited data set of 51 experimental runs, Figure S4.2 illustrates the predictive ability of the developed ANN tool to determine hydrogen yields from SSF hydrogen production of PMS without the need to conduct additional experimentation.

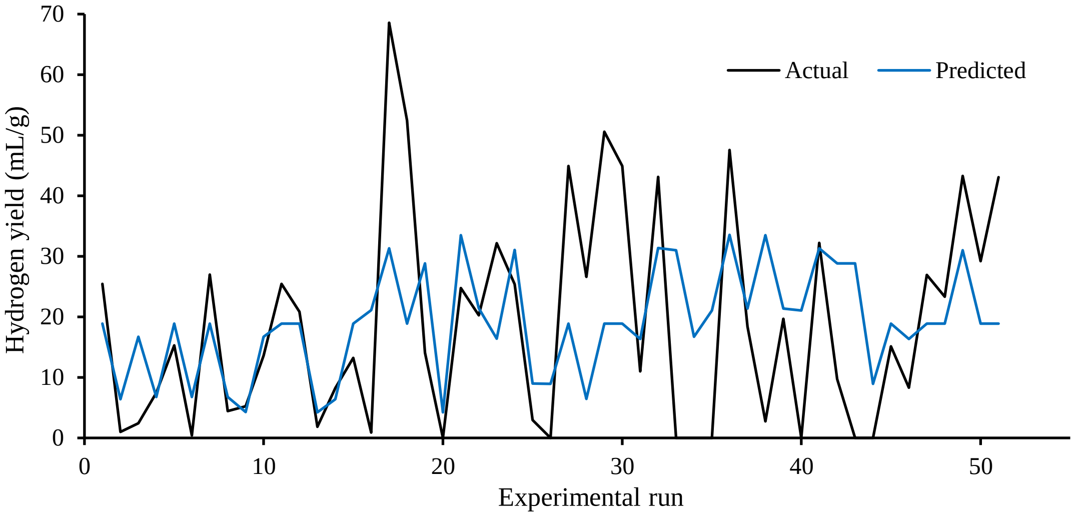


Figure S4.2: Line plot showing the predicted hydrogen yield values compared to the actual hydrogen yields obtained during the optimisation of SSF hydrogen production from PMS.

Regions in which the ANN tool correctly predicted the process output were observed between runs one and 14, with lesser predictive accuracy observed in conditions that generated very high or very low yields (outliers). The limited predictive capability of the predictive tool can be improved by retraining the model with additional experimental data. This data can be obtained by conducting additional experimentation or using previously published experimental data from studies focused on similar processes. Some of the limitations to using literature data includes; a lack of standardisation of data obtained from different researchers and incomplete process metrics such as yield and production rate.

CHAPTER FIVE

Enhanced methane potential from two-stage anaerobic co-digestion of paper mill sludge and pharmaceutical wastewater

This chapter has been submitted to *Current Research in Green and Sustainable Chemistry* with the title: Enhanced methane potential from two-stage anaerobic co-digestion of paper mill sludge and pharmaceutical wastewater.

Rorke, D. C. S*., Lekha, P., Kana, E. B. G. and Sithole, B. B. Enhanced methane potential from two-stage anaerobic co-digestion of paper mill sludge and pharmaceutical wastewater. Submitted and under review in *Current Research in Green and Sustainable Chemistry*.

5.1 Abstract

This study assessed single-stage and two-stage anaerobic digestion (AD) of paper mill sludge (PMS) using pharmaceutical wastewater (PW) as a novel nitrogen source. The single-stage AD process produced a low yield of 4.79 mL CH_4/gVS_{added}, indicating severe process instability. Using PW as the nitrogen source, simultaneous saccharification and fermentation from PMS during the first stage of two-stage anaerobic digestion (TSAD) gave a hydrogen yield of 33.56 mL H_2/gVS_{added}, while the second stage gave a yield of 28.36 mL CH_4/gVS_{added}. The modified Gompertz model displayed higher methane production rates and negligible lag times for TSAD. PW showed significant potential as an alternative to yeast extract for nitrogen supplementation, increasing the methane yield during TSAD by 1.83%. Compositional analysis of the resultant digestate showed promise for use as a soil amendment, satisfying South African fertiliser requirements. Results show the potential of PW as a waste-based source for nitrogen supplementation in TSAD of PMS. TSAD maximises energy recovery from PMS and provides an alternate fate for the waste stream, alleviating the financial burden of waste treatment and disposal experienced by the pulp and paper industry. Using digestate as a soil amendment could ensure nutrient recycling while mitigating the costs of AD residue disposal.

Keywords: two-stage anaerobic digestion, paper mill sludge, biohydrogen, biogas, biohythane, modified Gompertz

5.2 Introduction

A significant global dependence on fossil fuels for energy is not sustainable, as fossil fuel reserves are finite. The generation of greenhouse gases (GHGs) and their effect on the environment have driven researchers to develop sustainable and renewable energy solutions. An ever-increasing world population and global industrialisation have led to significantly increased levels of industrial waste generation. The treatment and disposal of these wastes often contribute significantly to environmental damage and the costs associated with production, as reported by Manfredi et al. (2009), who determined that conventional landfill systems directly contributed up to 300 kg CO_2-eq. tonne^{-1} towards GHG emissions. The generation of renewable fuels such as biogas, bioethanol, and biodiesel from waste can play a crucial role in reducing our dependence on fossil fuels and reducing the industrial carbon footprint.

Anaerobic digestion (AD) shows great potential as a method of generating energy from the treatment of organic waste, with the residual organic matter, called digestate, being used as a fertiliser (Achinas et al., 2017; Ragazzi et al., 2017). Furthermore, by capturing the biogas generated from AD systems, GHG emissions can be reduced. AD is a process in which anaerobic microorganisms break down organic material in a series of stages, namely hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Organic material is initially hydrolysed into soluble organic compounds such as sugars, long-chain fatty acids (LCFAs), and amino acids. The resultant material is then converted into volatile fatty acids (VFAs), hydrogen, ammonia, and carbon dioxide. Thereafter, these compounds are converted to precursors for methane formation, such as acetate, hydrogen, and carbon dioxide, which are finally converted to a biogas mixture containing methane (55-75%), carbon dioxide (25-45%), hydrogen (5-10%), and trace amounts of other gases (Kamali et al., 2016; Yeshanew et al., 2018; Sołowski et al., 2020).

AD can be performed as a single-stage process, where all four stages take place in one digestion vessel, referred to in this study as single-stage anaerobic digestion (SSAD). However, due to the variety of metabolic processes which occur during AD, process performance is often severely hampered by microbial instability, causing long hydraulic retention time (HRT) and low product yield (Akobi et al., 2016). Alternatively, hydrolysis and acidogenesis and acetogenesis can be carried out separately from methanogenesis in a two-stage process. Two-stage anaerobic digestion (TSAD) has gained renewed interest as it contributes to a more stable microbial environment, increased energy recovery, shorter HRT, and higher chemical oxygen demand (COD) removal (Lowe et al., 2019). This improvement is due to the variation in pH requirements of hydrolytic and acidogenic microorganisms and acetogenic and methanogenic microorganisms.

Microorganisms responsible for hydrolysis and hydrogen production exhibit optimum growth rates at a pH of 5.5 to 6.5, while acetogens and acetotrophic and acetoclastic methanogens are slower growing and exhibit optimal growth at pH 6.8 to 7.6 (Nkemka et al., 2015). Biohydrogen production by dark fermentation (DF) has garnered significant interest as a clean, renewable energy source (Dahiya et al., 2021). The combustion of hydrogen releases only energy and water. It is a clean fuel with a high energy density of 122 kJ/g that can be used for electricity generation (Rorke and Gueguim Kana, 2016). DF occurs under the principle of hydrolysis and acidogenesis of AD, with the methanogenic microorganisms often stressed by heat, pH, sonication of chemical pretreatment to inhibit methanogenesis (Sołowski et al., 2020). Some of the drawbacks of DF include low productivity and the formation of VFAs and alcohols (Detman et al., 2017). Efficiently utilising this effluent for methane production in a separate system would increase energy recovery and improve process performance. Some of the process conditions that influence SSAD and TSAD are inoculum source, pH, and carbon-to-nitrogen ratio (C/N). A C/N of 20 to 30 has been reported to enhance the stability of AD (Kalil et al., 2008; Sayara and Sánchez, 2019); however, to achieve an optimal C/N using waste streams, supplementation with organic nitrogen sources such as yeast extract (YE), peptone and casein or mineral nitrogen sources such as NH_4Cl and $(NH_4)_2SO_4$ is often required (Kalil et al., 2008; Hamilton et al., 2018).

In addition to the production of hydrogen and methane as separate energy carriers, biohythane, a mixture of hydrogen and methane, has emerged as an advanced automotive fuel. It contains 10-15% hydrogen, 50-55% methane, and 30-40% carbon dioxide and exhibits several advantages over methane or hydrogen individually, including a high flammability range, reduced ignition time and temperature, improved engine performance and reduced nitrous oxide emission. The co-production of hydrogen and methane achieved in TSAD is therefore gaining popularity as the preferred method for biohythane production (Hans and Kumar, 2019; Cremonez et al., 2021).

Although AD of pulp and paper mill wastewater is well established, AD of pulp and paper industry wastes such as paper mill sludge (PMS) is often hampered by long retention times due to incomplete hydrolysis of the lignocellulose and other organic materials present in PMS (Mahmood and Elliott, 2006; Veluchamy et al., 2018). Therefore, total energy recovery can be enhanced by implementing a TSAD process that can reduce digestion times by improving lignocellulose hydrolysis and VFA production in the first stage and providing an effluent rich in VFAs that is more amenable to AD for increased methane production during the second stage. Introducing a second waste stream that complements and balances the nitrogen deficiency exhibited by paper mill sludge (PMS) would, to some extent, alleviate the cost associated with nutrient supplementation. Effluents and wastewater generated from the production of high-quality amino acids and other pharmaceutical products exhibit low C/N, high salinity, and high levels of nitrogenous compounds (Shi et al., 2017). Using wastes high in N may therefore sufficiently contribute to the C/N balance required for optimal AD functionality. The implementation of a TSAD system for hydrogen and methane production using various substrate sources has been reported in the literature. The reports indicate significant potential to generate more energy than single-stage systems (Hernandez and Edyvean, 2011; Yang et al., 2011; Akobi et al., 2016; Detman et al., 2017; Lavagnolo et al., 2018; Lowe et al., 2019; Markphan et al., 2020). However, there is limited literature available on TSAD systems in PMS valorisation. Viñas et al. (1993) reported a 16% increase in process efficiency using thermomechanical pulp (TMP) wastewater using TSAD, while An et al. (2020) recently achieved a 50% increase in energy yield from paper sludge (PS) with *Clostridium thermocellum* augmentation during the first stage of TSAD.

The present study aimed to determine the potential of pharmaceutical wastewater (PW) as an N source for methane production from PMS in single-stage and two-stage AD systems. A two-stage process that combined hydrogen and methane production was developed. During the first stage, hydrogen was produced under dark fermentative, simultaneous saccharification and fermentation (SSF) conditions. The effluent of hydrogen production was then used as a substrate for methane production. The modified Gompertz equation was used to determine the kinetic parameters of the processes. Lastly, the resultant digestate from each process was assessed for potential use as a soil amendment.

5.3 Materials and Methods

5.3.1 Inoculum and culture media

All experimental tests were conducted using digestate from a lab-based anaerobic digester (volatile solids (VS) of 6.60% wet wt.) as the inoculum source. For the first stage of TSAD, the digestate was heat-treated at 121 °C, as Rorke et al. (2021) described. PMS from a local Kraft mill and PW from a local amino acid and pharmaceutical production company in South Africa were used as feedstock. PMS was air-dried and pretreated using a surfactant-assisted green liquor dregs (GLD) pretreatment described by Rorke et al. (2021). GLD was acquired from a local paper mill in South Africa, whereas Tween-80, cellulase from *Trichoderma reesei*, yeast extract, and ammonium nitrate were purchased from Sigma-Aldrich, South Africa. PW samples were stored at 4.0 °C before use. As described by Rorke and Gueguim Kana (2016), a sterile mineral salt solution was used in each process, without NH_4Cl, either during the first stage of TSAD or during SSAD.

5.3.2 Experimental set-up

Previously optimised and validated SSF hydrogen production process conditions (unpublished data) were used to carry out the first stage of TSAD. Standard organic and inorganic N sources were included as a comparison. The conditions used are shown in Table 5.1. Appropriate quantities of N sources were combined with PMS to achieve the optimised C/N ratios of 50, 30 and 50 for PW, ammonium nitrate and yeast extract (YE), respectively, and a volume of 75 mL sterile mineral salt solution was added. The fermentation flasks were inoculated at a concentration of 10% (v/v). The pH was adjusted to optimal values using 5 M NaOH or 1 M HCl, and cellulase was added thereafter. The working volume was made up to 150 mL using dH_2O, and each flask was flushed with N_2 gas for 60 s to obtain an anaerobic environment. All flasks were then incubated at 37 °C ±0.5 °C, at 80 rpm. Hydrogen production ceased after 66 h.

Table 5.1: Optimal SSF process conditions for hydrogen production from PMS

N source	**Enzyme dosage (FPU/g)**	**Substrate conc. (g/L)**	**pH**	**C/N**
PW	150.00	18.04	7.00	50
Ammonium nitrate	142.37	20.09	5.87	30
YE	150.00	29.18	7.00	50

To carry out the second stage of TSAD, the effluent obtained after hydrogen production was used as feedstock for methane production. For SSAD, PMS and appropriate quantities of N sources were added to each fermentation flask to obtain a C/N ratio of 25. A volume of 75 mL sterile mineral salt solution was then added. For processes in which C/N was re-adjusted to 25, appropriate quantities of N sources were added to the substrate. For TSAD processes in which C/N remained unadjusted, no additional N sources were added. All AD processes were conducted at an initial VS content of three to four grams. Fermentation vessels were inoculated at an inoculum-to-substrate (I:S) ratio of 2:1 on a VS basis, and pH was adjusted to 7.0 for all processes. The working volume was made up to 150 mL using dH_2O and flushed with N_2 gas for 60 s. All flasks were incubated at 37 °C ±0.5 °C and manually shaken once daily for 60 s. Fermentation flasks containing equivalent volumes of inoculum were used as controls to quantify background gas production.

5.3.3 Monitoring and analytical methods

Daily biogas volumes were determined using the water displacement method (Rorke et al., 2021), and the gas composition was analysed using a portable multi-gas analyser (SKZ Industrial, Shandong, China). The sensor determines the gas composition (CH_4, H_2) based on the principle of catalytic conversion or infrared absorption. The cumulative hydrogen and methane volumes produced were calculated using the equation (Eq. 5.1) described by Rorke et al. (2021).

$$V_{H,i} = V_{H,i-1} + C_{H,i}\left(V_{G,i} - V_{G,i-1}\right) + V_H\left(C_{H,i} - C_{H,i-1}\right) \tag{5.1}$$

Samples were taken from all fermentation flasks to determine VS, total solids (TS), pH, CHNS, major and trace element composition, and electrical conductivity. VS and TS were determined in duplicate by subjecting samples to 105 °C for TS content and then 550 °C for VS content until a constant weight was obtained. Hydrogen and methane yields are expressed as volume per gram of VS (mL/gVS$_{added}$). The pH and electrical conductivity were determined using pH and electrical conductivity sensors, and CHNS analysis was conducted using an Elementar Vario EL Cube elemental analyser (Elementar-Straße, Langenselbold, Germany). Major and trace elements were detected by inductively coupled plasma mass spectrometry (ICP-MS) at the Central Analytical Facilities at Stellenbosch University, South Africa, as described by Rorke et al. (2021). Data obtained from all processes were fitted using the modified Gompertz equation, using the least-squares method (Curve Expert V2.7.3, MyBiosource Inc, USA) to illustrate the efficiency of each process, shown in Equation 5.2.

$$G = G_m . \exp\left\{-\exp\left[\frac{R_m . \exp(1)}{G_m}(t_L - 1) + 1\right]\right\} \tag{5.2}$$

Where G is cumulative gas production (mL), G_m is maximum potential gas production (mL), R_m is maximum potential gas production rate (mL/h), and t_L is the time from the start of fermentation to exponential gas production (lag time) (h).

5.4 Results and Discussion

5.4.1 Characterisation of PW as an N source for AD of PMS

Table 5.2 illustrates the analysis of PW and PMS to determine the possible effects of the substrate composition on biogas production. The very high C/N of PMS shows the significant deficiency in N, which needs to be balanced in order for AD to be successful. In contrast, the much lower C/N exhibited by PW serves as an indicator of the potential use of PW as an N source. Elemental analysis conducted on PW determined macronutrient levels of 79.95, 1.80, 3.12, and 0.04 g/L for calcium (Ca), magnesium (Mg), potassium (K), and phosphorous (P), respectively, as well as high levels of sodium (Na) of 62.19 g/L. Additionally, PMS exhibited macronutrients Ca, Mg, K, and P at levels of 3.46, 0.64, 0.10, and 0.03 g/kg, respectively, with a relatively low Na level of 0.29 g/kg. The very high level of Na in PW poses a threat to microbial growth. Although Na is an obligatory growth requirement for microorganisms in the rumen (Caldwell and Hudson, 1974), increased concentrations can negatively affect AD. Zhang et al. (2017) reported a 90% inhibition of methane production during the AD of *Sargassum sp.* at a concentration of 18.70 gNa/L, suggesting the use of small volumes of PW to reduce the risk of inhibition. In addition, a study by Feijoo et al. (1995) assessed Na inhibition in AD, displaying a 50% inhibitory concentration range of 3.0 to 16.0 gNa/L in the absence of other salts and nutrients. Vintiloiu et al. (2012) detected Na at a concentration range of 0.36 to 6.04 g/kg in 25 operational anaerobic digesters, further substantiating a reduced inhibitory effect at low concentrations. Furthermore, Shi et al. (2017) reported a number of potential inhibitory compounds found in PW, including antibiotic residues, dissolved organic nitrogen, and residual solvents, which may significantly impair AD efficiency.

Table 5.2: Characteristics of PMS and PW for use as substrates in AD

	PMS	PW
pH	7.68	6.04
TS (%)	1.20	19.30
VS (wet wt. %)	55.20	11.39
VS (dry wt. %)	97.52	59.10
Ash (wet wt. %)	1.30	7.94
Ash (dry wt. %)	2.30	41.09
Carbon (%)	43.89	0.14
Nitrogen (%)	0.18	1.30
C/N	243.83	0.11

5.4.2 Biogas production from PMS and PW using a TSAD system

Cumulative hydrogen production and cumulative methane production of TSAD of PMS, using PW as an N source, can be seen in Figure 5.1. A maximum hydrogen volume of 131.88 mL was obtained during the first stage, corresponding to a yield of 33.56 mL H_2/gVS_{added}. Analysis of the effluent derived from DF was conducted to determine the shift in C/N after hydrogen production. The effluent from the PW-supplemented process exhibited a C/N of 33.87, and AD of this effluent gave a volume of 113.03 mL. Anaerobic digestion of the effluent, adjusted to a C/N of 25, resulted in a maximum of 115.69 mL methane, which corresponds to a yield of 28.36 mL CH_4/gVS_{added}. A slight increase of 8.89% in methane yield was observed when C/N was not adjusted due to a lower initial VS content. Additionally, without re-adjustment of the C/N using PW, no further Na was introduced into the system, reducing the risk of process inhibition.

A total yield of 33.56 mL H_2/gVS_{added} and 30.88 mL CH_4/gVS_{added} was thus obtained from the TSAD of PMS, using PW as an N source, without additional C/N adjustment. A cumulative yield of 14.70 mL CH_4/gVS was reported by Priadi et al. (2014) when paper sludge was used as a single substrate for AD, while a yield of 269 mL CH_4/gVS was achieved when paper sludge was combined with cow manure to obtain a more suitable C/N and higher initial VS content. This, therefore, suggests that an increase in the initial VS content of an AD system will allow for increased yields.

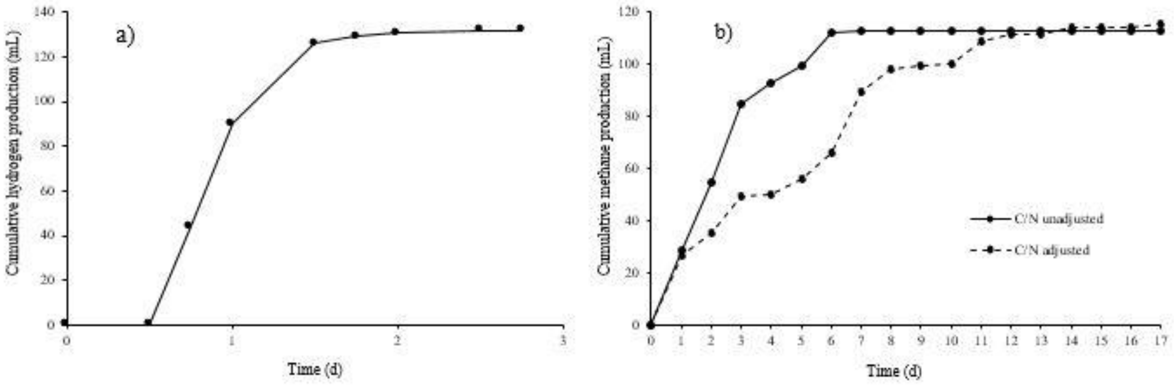


Figure 5.1: Cumulative volumes obtained during TSAD of PMS using PW as N source for a) hydrogen production and b) methane production

5.4.3 Biogas production from PMS and PW using an SSAD system

Daily cumulative methane volumes obtained from the AD of PMS, using PW, YE, and ammonium nitrate as N sources were 20.39, 29.67, and 18.58 mL after background gas production from the control was deducted. Reactors were monitored until daily biogas production ceased at ten days. A peak in methane volume produced was observed on the third day for SSAD processes supplemented with PW or YE as an N source, while the process supplemented with ammonium nitrate exhibited a slower start and peaked on the fourth day. Ammonium nitrate as an inorganic N source showed a significantly lower process efficiency, exhibiting a yield of 4.67 mL CH_4/gVS_{added}, even lower than the yield of 4.79 mL CH_4/gVS_{added} when using PW. In comparison, a yield of 7.79 mL CH_4/gVS_{added} was obtained using YE as an N source. YE is known to enhance the growth of methanogenic microorganisms (Wagner et al., 2012). This may be due to the number of compounds it contains, which are of nutritional value, including amino acids, vitamins, and minerals (Hakobyan et al., 2012). A similar effect was observed by Kalil et al. (2008), where the hydrogen yield obtained was 50% lower than that obtained using YE, illustrating the nutritional deficiency of ammonium nitrate. Additionally, Liu et al. (2017) reported a mechanism of hydrogen consumption due to nitrate or nitrate reduction, leaving less hydrogen available for methane production. Ammonium nitrate was therefore excluded for the remainder of the study.

5.4.4 Effect of PW as N source on methane production

The implementation of N sources such as YE as a complex organic N source is widely accepted. The cost of supplementary reagents can be alleviated using other waste streams high in N. This can be observed in Figure 5.2, where the volume of methane achieved between YE and PW is comparable in both the C/N adjusted and unadjusted TSAD systems. Effluent obtained after DF exhibited C/N values of 33.87 and 38.62 for PW and YE, respectively, as well as a pH value of 4.76, indicating increased fatty acid concentrations. At these ratios, TSAD of PMS with PW resulted in a 7.33% increase in methane yield compared to that of YE. A similar increase in methane volume was observed in both processes until the third day of digestion, and while methane production plateaued at a volume of approximately 100 mL in the case of YE, a further increase to 112 mL was observed until the sixth day of digestion for PW, with slight increases until day 17. Effluent from DF exhibited relatively balanced C/N ratios, but a further adjustment to 25 resulted in an 18.26% increase in methane volume produced for the process supplemented with YE, reaching a maximal methane volume of 124.50 mL after 17 days of incubation. A significantly smaller increase in methane volume of 2.66 mL was observed in the PW supplemented process. This observation can be explained by the second addition of YE increasing the available nutrients for microbial growth and methane production, while further increasing the PW in the system may lead to increased Na levels that may exhibit inhibitory effects on methanogenesis. Nevertheless, a 1.83% increase in methane yield was observed when PW was used for C/N adjustment, as YE increased the VS content of the system to a greater extent than that of PW.

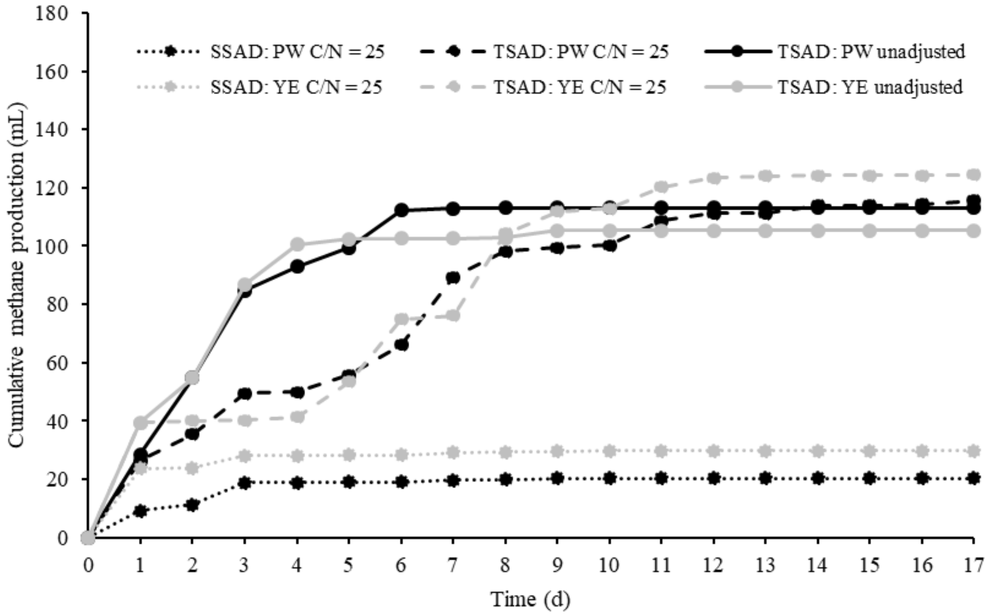


Figure 5.2: Cumulative methane production obtained during AD of PMS, using PW and yeast extract as N sources

Cumulative biogas data were fitted using the modified Gompertz model to determine the biogas production potential of each system. The kinetic parameters obtained are shown in Table 5.3. High R^2 values indicate a good fit with the biogas data, illustrating that the modified Gompertz model described at least 95% of the variation observed in the data. A study by Pramanik et al. (2019) described the superiority of the modified Gompertz model to the logistic function and first-order kinetic models regarding the capability of fitting experimental data. A significant variation in process kinetics for PW and YE was observed in SSAD of PMS, with YE supplementation resulting in a much higher maximum potential gas production (G_m) of 28.33 mL, as opposed to 19.90 mL for PW. This correlated with a higher maximum potential gas production rate (R_m) observed for YE supplemented processes.

Interestingly, a negative lag time (t_L) observed for SSAD using PW suggests that a very short lag time was observed and is thus negligible, while a lag time of 2.97 h was determined for SSAD of PMS using YE. Predicted maximum methane volumes displayed 2.40 and 4.73% variation compared with measured volumes for PW and YE, respectively. For the TSAD systems, adjustment of C/N to 25 resulted in both the PW and YE supplemented systems displaying the same R_m of 0.5 mL/h, with the predicted maximum methane volume of 135.89 mL for YE exhibiting 7.15% variation to the obtained volume of 124.50 mL. In contrast, the modified Gompertz model could more accurately predict the maximum methane volume obtained for the PW supplemented system, showing 3.47% variation. As methane production started less than a day after processes were initiated, negative lag times were predicted and are therefore negligible (Zwietering et al., 1992).

Table 5.3: Modified Gompertz parameters determined for AD of PMS using PW and YE

	TSAD								**SSAD**			
	C/N unadjusted				**C/N 25**				**C/N 25**			
	G_m (mL)	R_m (mL/h)	t_L (h)	R^2	G_m (mL)	R_m (mL/h)	t_L (h)	R^2	G_m (mL)	R_m (mL/h)	t_L (h)	R^2
PW	113.28	1.28	4.25	0.99	119.70	0.50	-12.09	0.98	19.90	0.29	-1.01	0.96
YE	104.93	1.40	1.71	0.98	135.89	0.50	-10.26	0.95	28.33	1.29	2.97	0.97

Kinetic data observed for TSAD systems using DF effluent without C/N adjustment showed a higher predicted maximum methane volume of 113.28 mL for the PW supplemented system, displaying a slight variation of 0.22% from the observed methane volume. A slightly lower R_m of 1.28 mL/h and a higher lag time of 4.25 h suggests a slower AD process than the YE system. A higher yield observed in the PW system may be due to a contribution from PW to the methanogenic microbial community available in the AD system (Shi et al., 2017). A maximal yield of 90 mL CH_4/gCOD was reported by Bakraoui et al. (2020) using recycled pulp and paper sludge in SSAD, illustrating the potential benefit of TSAD systems to enhance biogas yields in the form of hydrogen and methane.

5.4.5 Assessment of digestate properties for use as a soil amendment

To enhance the optimal utilisation of nutrients, recycling digestate to agricultural fields for nutritional enrichment remains one of the environmentally friendly disposal methods for digestate from AD (Westphal et al., 2016). Compared to the original composition of substrates used for AD, the digestion process results in a number of compositional changes observed in the resulting digestate (Möller and Müller, 2012). Table 5.4 presents the compositional makeup of the digestates obtained after TSAD and SSAD of PMS, using PW and YE as N sources. All resultant digestate streams exhibited high pH values between 8.60 and 9.80, mostly falling into the range described by Möller and Müller (2012) for typical digestate. The high pH of digestate is primarily due to the reduction in fatty acids, the production of ammonium carbonate, and the removal of CO_2 during methane production (Möller and Müller, 2012). A low C/N of 8.05 exhibited by the digestate obtained from TSAD of PMS and PW at an initial C/N of 25 illustrates the significant increase in N-containing compounds during AD, corresponding to the higher methane production observed, compared to the SSAD of PMS and PW. The higher C/N of 15.13 exhibited by the same process using YE suggests a lower production of nitrogenous compounds and a lower risk of inhibition by ammonia build-up. Electrical conductivity (EC) is often used to indicate salinity levels. The EC obtained by TSAD of PMS and PW and TSAD of PMS and YE at C/N of 25 exceeded the suggested limit of 5.00 mS/cm for digestates to be used as soil amendments without dilution (Valentinuzzi et al., 2020). Möller and Müller (2012) suggest a digestate:water dilution range of 1:4 to 1:8 to reduce EC.

Table 5.4: Compositional analysis of digestate obtained from TSAD and SSAD of PMS compared to previous studies

	PW			YE					
	TSAD		SSAD	TSAD		SSAD	Reference		
	C/N = 25	C/N unadjusted	C/N = 25	C/N = 25	C/N unadjusted	C/N = 25	Möller and Müller, 2012	Valentinuzzi et al., 2020	Barłog et al., 2020
pH	8.61	9.28	8.75	9.80	9.24	9.00	7.30-9.00	8.77	8.53
TS (%)	3.75	2.89	3.27	2.63	2.69	2.82	1.50-13.20	8.80	5.83
VS (% of TS)	60.81	73.06	78.75	77.04	78.81	77.75	63.80-75.00	-	-
C (g/kg)	306.00	391.45	410.10	422.10	375.60	404.70	36.00-45.0[a]	36.00[a]	-
H (g/kg)	51.00	49.25	40.50	59.80	48.10	47.10	-	-	-
N (g/kg)	38.00	22.45	22.20	27.90	28.70	15.10	3.10-14.00[a]	8.40[a]	31.40
S (g/kg)	2.30	2.35	2.40	3.70	2.40	1.90	-	-	4.89
C/N	8.05	17.4	18.64	15.13	13.09	26.80	3.00-8.00	4.20	-
EC (mS/cm)	18.50	6.90	4.82	6.80	4.90	3.65	-	4.60	-
Macronutrients (g/kg)									
P	7.87	8.12	9.78	16.98	6.73	13.86	6.00-17.00	4.30[a] as P_2O_5	11.70
K	16.98	14.35	19.71	45.53	15.04	26.65	19.00-43.00	10.70[a] as K_2O	55.90
Ca	66.55	10.86	15.05	13.30	7.60	12.69	-	-	29.60
Mg	4.31	2.71	3.22	4.18	2.44	4.03	0.30-0.70	3.60[a] as MgO	8.24
Trace elements (mg/kg)									
Cu	36.30	429.91	412.78	65.44	34.44	50.85	-	10.00	90.10
Fe	2203.07	5041.24	5255.51	3004.33	1718.06	3400.00	-	0.25[a]	2962.00
Mn	639.85	773.20	867.84	948.48	533.04	1010.00	-	360.00	285.70
Zn	168.15	187.13	217.22	326.96	147.17	326.00	-	135.00	295.00
Heavy metals (mg/kg)									
As	0.47	0.50	0.57	0.89	0.54	0.73	-	-	-
Cd	0.15	0.17	0.12	0.25	0.13	0.22	-	0.10	-
Pb	2.08	2.06	1.43	3.67	1.80	2.18	-	11.00	-
Hg	0.10	0.08	0.08	0.16	0.07	0.13	-	0.20	-

[a]: % dry matter.

Macronutrient content determined for all digestates showed similarities to previous studies, except for Ca in digestate obtained from TSAD of PMS and PW at C/N adjusted to 25. This may be due to the high Ca content of PW (unpublished data), which was added to the DF effluent for C/N adjustment. Dahunsi et al. (2021) assessed the effect of digestate containing 89.06 gCa/kg obtained from the AD of papaya peels on crop performance and soil fertility and found it to outperform NPK 15-15-15 fertiliser. This suggests that the high Ca content observed will not negatively affect the performance of the digestate as a soil amendment. All digestates obtained satisfy the requirements of a sum of N, P, and K content of at least 40 g/kg for organic fertiliser mixtures in South Africa, described in the South African Act No. 36 of 1947 for fertilisers (Department of Agriculture, Forestry and Fisheries, 2017). Although South Africa does not regulate heavy metals in fertilisers, the heavy metals in the digestates obtained in this study were detected at relatively low concentrations.

5.5 Conclusions

This study assessed a two-stage system for AD of PMS using PW and YE as N sources. The TSAD of PMS exhibited advantages over the standard single-stage system, including enhanced methane yields, increased process stability, and efficiency. Furthermore, PW as an alternative N source to YE showed significant potential. A maximum hydrogen yield of 33.56 mL H_2/gVS_{added} was obtained using PW as the N source for C/N adjustment of the carbon-rich PMS. A 6-fold increase in methane yield was obtained when SSAD was replaced with TSAD, using PW, while a 1.83% increase in methane yield was observed using PW instead of YE in the TSAD system. Kinetic assessment using the modified Gompertz model displayed high maximum methane production rates and negligible lag times in TSAD.

Additionally, analysis of the composition of the resultant digestate suggests that it can be applied to soil as a conditioner as it satisfies the criteria of the South African Act No. 36 of 1947 for fertilisers. This will allow for nutrient recycling, reducing the disposal costs of digestate derived from the AD of PMS and PW. A TSAD system for PMS and PW produces hydrogen and 28.36 mL CH_4/gVS_{added} and can thus be implemented to ensure maximal energy recovery of PMS. The composition of the resultant digestate suggests that it can be applied to soil as a conditioner.

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CHAPTER SIX

Conclusions and Recommendations

6.1 Conclusions

Access to electricity has the potential to improve quality of life significantly. However, the growing global dependence on finite and rapidly depleting fossil fuels for energy has rapidly increased energy demands which are becoming more difficult to meet. Furthermore, the persistent use of fossil fuels exacerbates environmental pollution. The development of green, sustainable energy practices has therefore gained interest. Bioenergy research is plagued with low product yields, high process costs and prolonged fermentation durations. The valorisation of waste streams for bioenergy production offers significant environmental and economic advantages. As a lignocellulosic waste stream from the pulp and paper industry, PMS is a valuable resource for the production of biofuels and value-added products; however, the presence of ash, heavy metals, and other process rejects are an obstacle to efficient conversion.

This study was therefore aimed at the treatment and beneficiation of PMS by; developing a low-cost pretreatment protocol to improve the digestibility of PMS, producing valuable biofuels such as hydrogen and methane from the pretreated PMS and assessing the feasibility of using the resultant digestate as a soil amendment. The conclusions of this study are summarised and discussed below, with reference to the aims and objectives outlined in section 1.5.

I. Develop a pretreatment protocol to enhance the fermentable sugar release and, thus, the digestibility of PMS.

A surfactant-assisted GLD pretreatment was developed to improve the release of fermentable sugar. Without pretreatment, enzymatic hydrolysis of PMS produced 12.34 g/L reducing sugar (RS), corresponding to a yield of 0.28 gRS/gPMS. This was improved to 16.38 g/L, giving a yield of 0.33 gRS/gPMS, using optimised conditions obtained from a combined mixture factorial RSM design. The developed pretreatment protocol also resulted in cellulose recovery, hemicellulose removal and lignin removal of 58.11, 46.78 and 67.84%, respectively. Furthermore, a significant reduction of at least 74% in the elements of aluminium, chromium, cobalt, nickel, cadmium, tin, lead and mercury was observed after pretreatment.

PMS was assessed as a substrate for hydrogen production to determine the link between increased reducing sugar concentrations obtained and digestibility. Untreated PMS had a negative effect on the microbial community and could not support the production of hydrogen without enzymatic hydrolysis, which produced 2.49 mL H_2/gPMS. A 48.59% improvement in the hydrogen yield was observed when PMS was screened and pretreated using the developed protocol before SSF hydrogen production. Subsequent hydrogen production was therefore conducted using an SSF concept.

II. Determine the effect of introducing a second waste stream as a nitrogen source during simultaneous saccharification and fermentation of PMS for hydrogen production.

The role of C/N in hydrogen production is often overlooked. Using OVAT, optimal C/N ratios of pharmaceutical wastewater (PW) and two traditional nitrogen sources, namely yeast extract and ammonium nitrate, were determined to be 50, 50 and 30, respectively. These ratios were then used throughout process optimisation. Utilising PW as a secondary waste stream to supplement the nitrogen deficiency of PMS during the optimisation of SSF hydrogen production gave a maximal hydrogen yield of 54.79 mL/gPMS, exhibiting a 22-fold improvement on yields obtained during preliminary assessment. In addition, PW performed similarly to yeast extract, which has been reported to contain additional growth factors that support microbial growth. A minor variation of 2.26% was observed between yields obtained using PW and yeast extract.

Kinetic studies exhibited coefficient of determination (R^2) values of 0.99, which indicated that the models could adequately describe the relationship between operational input conditions. Furthermore, data obtained illustrated that, although PW did not perform as well as yeast extract, a complex, organic nitrogen source, it gave 30.25 and 8.72% higher H_m and R_m values than ammonium nitrate, as well as a 48.33% lower lag time (t_L).

The accumulation of organic acids was monitored by the pH change throughout the hydrogen production process, with all processes reaching a pH value between 4.76 after the 66 h process duration. The acidification of the fermentation medium served as an indicator of organic acid accumulation, which severely hampered further hydrogen production. The acidified effluent was then directed toward anaerobic digestion for methane production.

III. Assess the feasibility of using the effluent from hydrogen production for methane production by AD.

Two-stage anaerobic digestion (TSAD) produced up to seven-fold higher methane yields than single-stage anaerobic digestion (SSAD). The effluent generated from the PW-supplemented hydrogen process gave a maximal methane yield of 30.88 mL/gVS, which was 7.33% higher than that of yeast extract. Furthermore, readjustment of the effluents C/N ratio to 25 produced higher cumulative volumes but negatively affected methane yields, illustrated by 8.35 and 3.20% lower yields for PW and yeast extract, respectively. In addition, process times were increased from 10 days to 17 days for TSAD processes adjusted to a C/N of 25, without a significant increase in yield. The modified Gompertz models of unadjusted TSAD processes exhibited higher production rates (Rm) of 1.28 and 1.40 mL/h for PW and yeast extract, respectively, whereas 0.50 mL/h was observed for TSAD systems readjusted to 25. This illustrates that there is no need to re-adjust the C/N of effluent generated from hydrogen production. In addition, a 7.96% higher cumulative methane production can be expected from the effluent of the PW-supplemented process than that of yeast extract.

IV. Determine the potential of using the resultant digestate as a soil conditioner.

Recycling digestate by utilising it as a soil amendment can potentially enhance the optimal use of nutrients. The resultant digestate generated from TSAD of PMS displayed typical pH values between 8.60 and 9.80 and C/N ratios between 8.00 and 17.40. In the case of the TSAD process readjusted to C/N of 25 using PW, an EC of 18.50 mS/cm indicated the increased salinity of PW, highlighting the need to use industrial waste streams at low volumes to mitigate process inhibition. The elements of heavy metals detected in the digestates were below 1.00 mg/kg, except for Pb, which was between 1.40 and 3.70 mg/kg.

Furthermore, all digestates contained the elements required for use as a soil amendment and satisfied the requirements outlined by the South African Department of Agriculture, Forestry and Fisheries, of a sum of N, P and K of at least 40g/kg for organic fertilisers.

6.2 Recommendations

The objectives of this study, to treat and beneficiate PMS by the production of hydrogen and methane were achieved. In the form of hydrogen and methane, bioenergy production from PMS can contribute to achieving a low-carbon economy by redirecting waste that is destined for landfills to more sustainable treatment practices. However, further research on increasing bioenergy yields is needed to improve the economic feasibility of PMS beneficiation if implemented at an industrial scale

It is therefore recommended that the following should be investigated in future studies:

- Conduct an in-depth analysis of the origin of elements of heavy metals and macronutrients detected in PMS.

The bio-availability of these elements may be influenced by their origin, and identifying the form in which they are present can significantly contribute to understanding the way PMS differs from conventional lignocellulosic and organic industrial wastes. Furthermore, the presence of inert compounds may hamper enzyme interaction and reduce the digestibility of PMS.

- Develop a lignin removal protocol for PMS.

Although previous literature suggests that PMS contains little to no lignin, a lignin content of 40.05% was determined. Developing a method to remove lignin from PMS while preserving the integrity of the lignin would provide an additional revenue stream. The remaining PMS can then be hydrolysed enzymatically.

- Determine the effect of additives such as surfactants on SSF hydrogen production

Surfactants have been shown to improve pretreatment and enzyme hydrolysis efficiency. Including a surfactant during hydrogen production as well may provide an enhanced effect on the process yield.

- Assess the feasibility of including a cellulosic enzyme during anaerobic digestion.

PMS digestibility is significantly enhanced in the presence of an enzyme. The effect of enzyme inclusion on methane production may produce more desirable results than the inclusion of an enzyme during hydrogen production only.

- Assess bioenergy production potential of PMS using fermenters with improved mixing capability

Due to the high water holding capacity of PMS, inferior mixing using shake-flasks may have negatively affected product yields. A possible solution to the low mixing efficiency of shake flasks would be to test the use of large-scale fermenters with impellers suited to viscous mixtures or develop miniaturised fermenters at volumes similar to that of shake flasks to enable the researcher to conduct numerous experiments at once.

- Conduct a techno-economic analysis on the pretreatment and two-stage anaerobic digestion of PMS.

A techno-economic study is required to determine the feasibility of beneficiating PMS using the optimised pretreatment protocol and TSAD.

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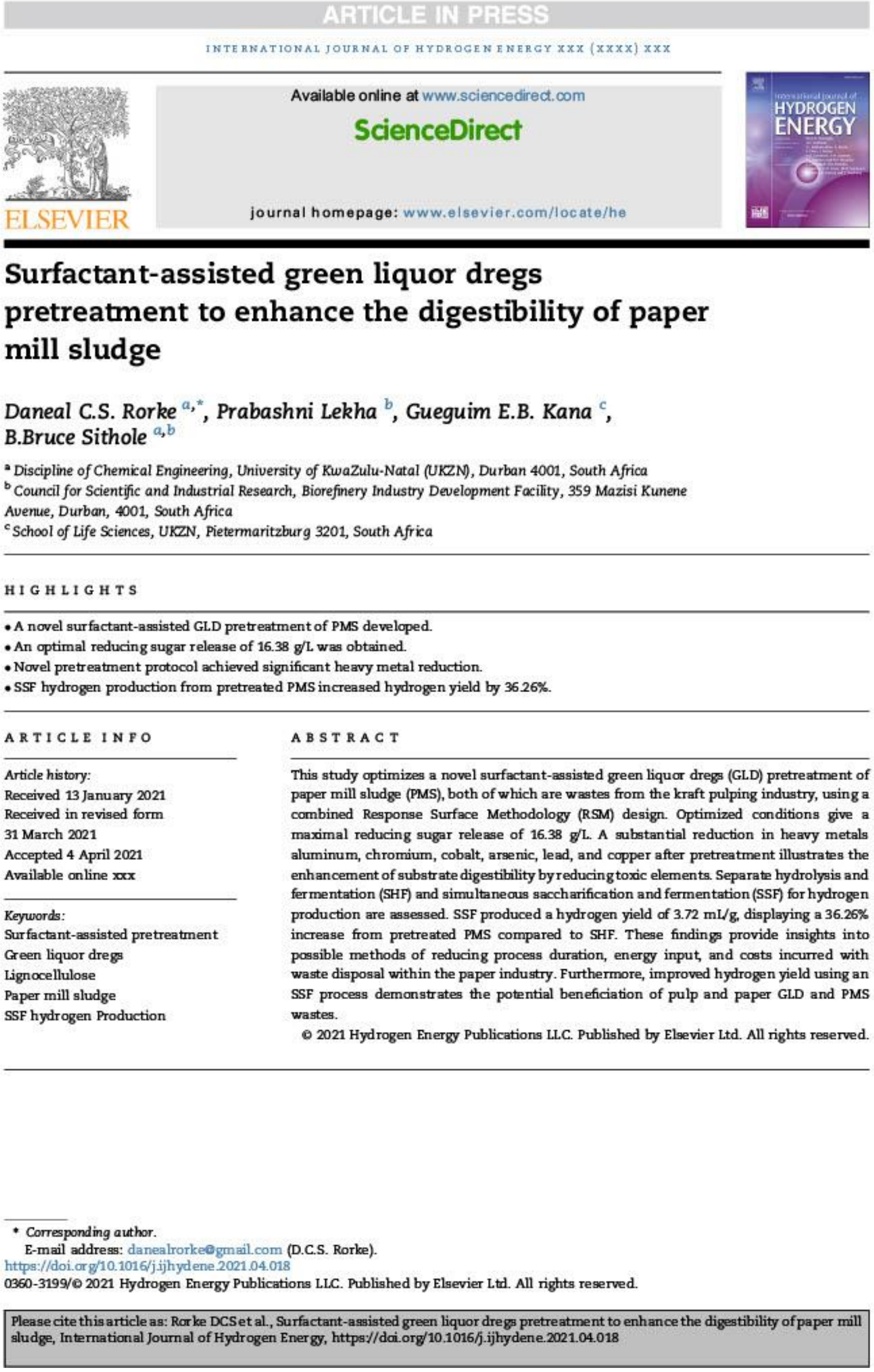

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Surfactant-assisted green liquor dregs pretreatment to enhance the digestibility of paper mill sludge

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HIGHLIGHTS

- A novel surfactant-assisted GLD pretreatment of PMS developed.
- An optimal reducing sugar release of 16.38 g/L was obtained.
- Novel pretreatment protocol achieved significant heavy metal reduction.
- SSF hydrogen production from pretreated PMS increased hydrogen yield by 36.26%.

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ABSTRACT

This study optimizes a novel surfactant-assisted green liquor dregs (GLD) pretreatment of paper mill sludge (PMS), both of which are wastes from the kraft pulping industry, using a combined Response Surface Methodology (RSM) design. Optimized conditions give a maximal reducing sugar release of 16.38 g/L. A substantial reduction in heavy metals aluminum, chromium, cobalt, arsenic, lead, and copper after pretreatment illustrates the enhancement of substrate digestibility by reducing toxic elements. Separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) for hydrogen production are assessed. SSF produced a hydrogen yield of 3.72 mL/g, displaying a 36.26% increase from pretreated PMS compared to SHF. These findings provide insights into possible methods of reducing process duration, energy input, and costs incurred with waste disposal within the paper industry. Furthermore, improved hydrogen yield using an SSF process demonstrates the potential beneficiation of pulp and paper GLD and PMS wastes.

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Introduction

With the introduction of a green economy, tighter environmental regulations have placed immense pressure on industries to valorize the various waste streams generated. The water and energy-intensive pulp and paper industry has been reported as the fifth largest global energy consumer [24], producing over 400 million tonnes of paper and cardboard each year [4,49]. Thus, this industry requires between 300 and 2600 m^3 water per tonne printing and writing paper produced [25] and uses up to 4% of the total global energy supply [24]. Therefore, the necessity to implement water recycling protocols has led to waste streams more concentrated in impurities and unrecyclable chemicals. PMS is one of the main wastestreams generated by the pulp and paper industry, with up to 40–50 kg dry sludge generated per tonne of paper produced [3]. The South African pulp and paper industry, in particular, generates up to 500 000 wet tonnes of PMS each year [6]. The cellulose-rich nature of PMS makes this waste a promising source for renewable energy production; however, the presence of impurities, fillers, and clay used during the paper-making process may negatively affect process economics.

Reduced fossil fuel resource availability has also led to increasing energy costs, making it difficult for smaller pulp and paper mills to remain economically viable. Furthermore, landfilling is quickly becoming an unacceptable and costly disposal method, leading to an increased interest in developing low-cost alternative energy production methods [49] and waste valorization. The valorization of PMS would therefore reduce or even eliminate the need for disposal and allow for increased revenue streams by the production of value-added products such as bioethanol, biogas, and biohydrogen, with biogas (50–55.5 MJ/kg) and biohydrogen (141.8 MJ/kg) displaying higher calorific values than bioethanol (29.78 MJ/kg) [47,48]. PMS has the additional benefit of reduced lignin content due to the delignification process carried out during paper-making, compared with other lignocellulosic biomass residues such as post-harvest waste, making the PMS less recalcitrant to microbiological processing [6]. Furthermore, the use of PMS as a feedstock will allow for significant savings regarding transport and disposal costs, while incorporating bioenergy production systems into existing paper mills will significantly reduce infrastructure and energy costs, with the possibility of an additional revenue stream.

Unfortunately, PMS is an industrial waste, and there are several disadvantages associated with its use as a potential renewable energy feedstock. The high water-holding capacity (WHC) of PMS (>0.3 cm^3/g substrate) often leads to a highly viscous fermentation medium, reducing mixing efficiency and mass transfer [6,7,65]. Furthermore, the high ash content in PMS, particularly that of recycled paper mills, imposes a negative effect on enzymatic hydrolysis due to the irreversible binding of enzymes to ash [9], leading to reduced cellulose breakdown and lower product yields. Additionally, the cost-intensive nature of enzyme usage calls for reduced enzyme loading to keep the process economically viable. Attempts to overcome these challenges and thus increase the amenability of PMS to microbial processes by implementing a variety of pretreatment protocols have been reported. Gurram et al. [27] reported a reduction in ash content of sludge from a kraft pulp and paper mill, from 32.2 to 11% employing chemical de-ashing using hydrochloric acid. This reduction in ash translated to a significant increase in cellulose conversion, from 14.9 to 75.6% after enzymatic hydrolysis. An increase in ethanol production, from 45 g/L to 60 g/L was achieved by Ref. [31] by de-ashing PMS before fed-batch simultaneous saccharification and fermentation (SSF). While [27] reported an increase in enzyme hydrolysis performance of 1.8 g/L to 25.9 g/L using 1% hydrogen peroxide pretreatment before enzymatic hydrolysis of de-ashed PMS [76]. Also reported improved PMS conversion rates, from 45.5 to 56.3 and 55.4% using non-ionic surfactants Triton X-100 (1%) and PEG 8000 (5%), respectively. Therefore, a pretreatment technique suitable to PMS must be developed to obtain the maximum possible fermentable sugar yields while ensuring that the process remains energy and cost-efficient.

wAlthough many pretreatment methods have been developed, the most commonly employed pretreatment regimes use alkali conditions to effectively remove lignin from the remaining cellulose and hemicellulose components of lignocellulosic biomass [20]. The solubilization and subsequent removal of lignin using alkaline pretreatment occur via ester bond degradation and glycosidic linkage cleavage, which leads to an altered lignin structure, lignin-hemicellulose complex reduction, and partial cellulose decrystallization [10]. Advantages of alkaline pretreatment include low operational costs, reduced holocellulose degradation, and minimized fermentation inhibitor production [10]. Residual cellulose and hemicellulose in a solid-state are then more amenable to enzymatic attack, resulting in enhanced fermentable sugar production for further value-added product development. Typically, hydroxides such as NaOH and KOH are used during alkaline pretreatments; however, the formation of salts leads to high costs and low sugar recovery [38], illustrating the need for cheaper alkali reagents.

Green liquor dregs (GLD) have recently received attention as a cheap and abundantly available alkaline pretreatment agent. GLD is an alkaline waste material generated by sulfate pulp and paper mills during the purification of green liquor. The suspended solids fraction (0.6–2.0 g/L) is generally made up of sodium carbonate, calcium carbonate, calcium phosphate, sodium hydroxide, sulfides, unburned carbon, and trace amounts of heavy metals [23]. GLD exhibits a high pH of 11, creating possibilities of its use as an alkaline barrier at landfill sites or as a liming agent. Green liquor has been reported as a pretreatment agent for lignocellulosic biomass [30,53]. Additionally [54] illustrated the use of GLD as a neutralizing agent for acidic wastewaters; however, GLD is not as commonly used as a pretreatment agent, and there is limited knowledge of its use for PMS.

Furthermore, the use of surfactants such as sodium dodecyl sulfate (SDS), Triton X-100, polyethylene glycol (PEG), and Tween-80 as an enhancement technique during pretreatment has been reported [51,61,66]. [75] reported that non-ionic surfactant Tween-80 enhanced enzymatic hydrolysis by swelling lignocellulosic fibers, increasing surface area, and

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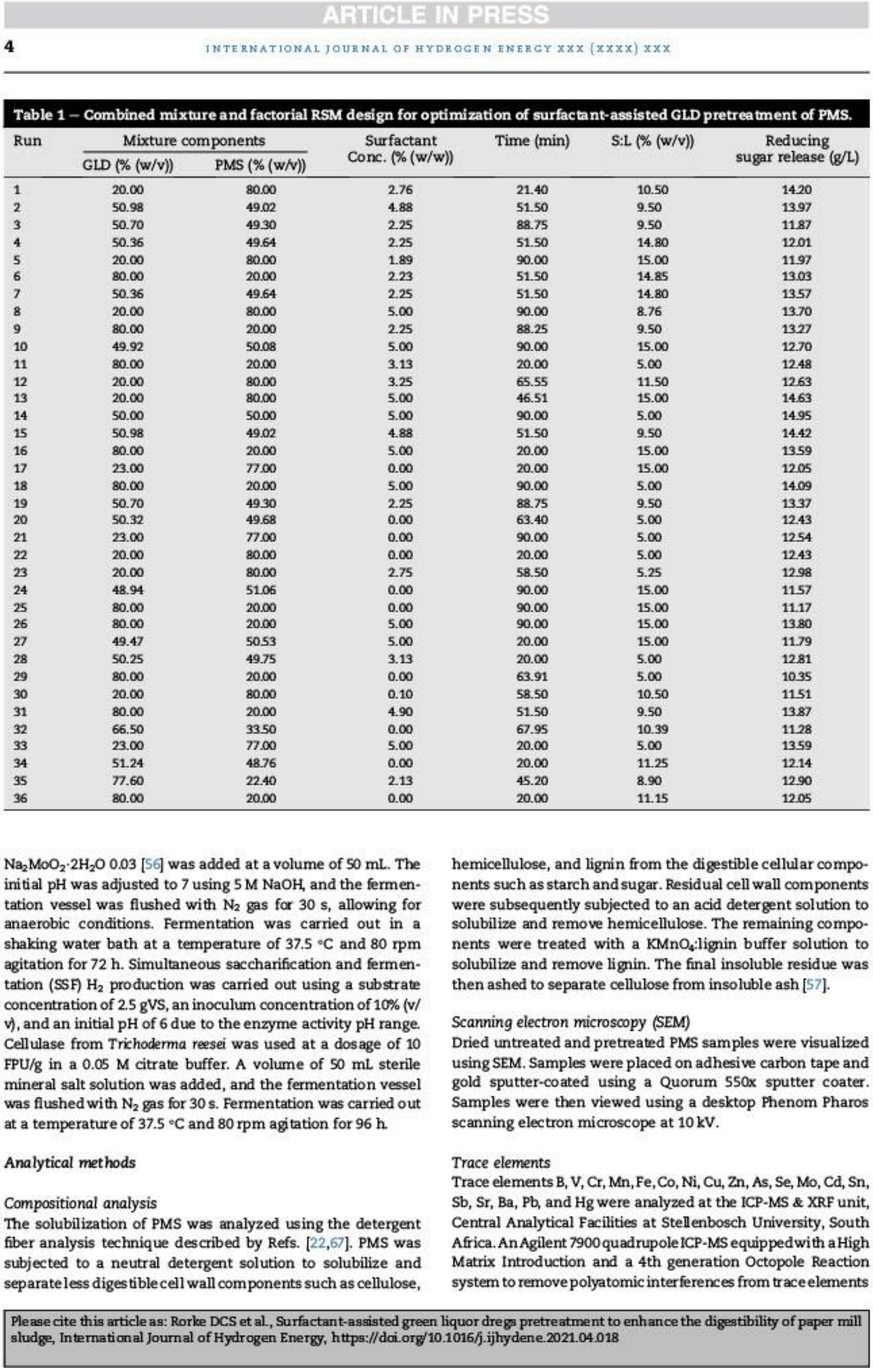

Table 1 – Combined mixture and factorial RSM design for optimization of surfactant-assisted GLD pretreatment of PMS.

Run	Mixture components		Surfactant Conc. (% (w/w))	Time (min)	S:L (% (w/v))	Reducing sugar release (g/L)
	GLD (% (w/v))	PMS (% (w/v))				
1	20.00	80.00	2.76	21.40	10.50	14.20
2	50.98	49.02	4.88	51.50	9.50	13.97
3	50.70	49.30	2.25	88.75	9.50	11.87
4	50.36	49.64	2.25	51.50	14.80	12.01
5	20.00	80.00	1.89	90.00	15.00	11.97
6	80.00	20.00	2.23	51.50	14.85	13.03
7	50.36	49.64	2.25	51.50	14.80	13.57
8	20.00	80.00	5.00	90.00	8.76	13.70
9	80.00	20.00	2.25	88.25	9.50	13.27
10	49.92	50.08	5.00	90.00	15.00	12.70
11	80.00	20.00	3.13	20.00	5.00	12.48
12	20.00	80.00	3.25	65.55	11.50	12.63
13	20.00	80.00	5.00	46.51	15.00	14.63
14	50.00	50.00	5.00	90.00	5.00	14.95
15	50.98	49.02	4.88	51.50	9.50	14.42
16	80.00	20.00	5.00	20.00	15.00	13.59
17	23.00	77.00	0.00	20.00	15.00	12.05
18	80.00	20.00	5.00	90.00	5.00	14.09
19	50.70	49.30	2.25	88.75	9.50	13.37
20	50.32	49.68	0.00	63.40	5.00	12.43
21	23.00	77.00	0.00	90.00	5.00	12.54
22	20.00	80.00	0.00	20.00	5.00	12.43
23	20.00	80.00	2.75	58.50	5.25	12.98
24	48.94	51.06	0.00	90.00	15.00	11.57
25	80.00	20.00	0.00	90.00	15.00	11.17
26	80.00	20.00	5.00	90.00	15.00	13.80
27	49.47	50.53	5.00	20.00	15.00	11.79
28	50.25	49.75	3.13	20.00	5.00	12.81
29	80.00	20.00	0.00	63.91	5.00	10.35
30	20.00	80.00	0.10	58.50	10.50	11.51
31	80.00	20.00	4.90	51.50	9.50	13.87
32	66.50	33.50	0.00	67.95	10.39	11.28
33	23.00	77.00	5.00	20.00	5.00	13.59
34	51.24	48.76	0.00	20.00	11.25	12.14
35	77.60	22.40	2.13	45.20	8.90	12.90
36	80.00	20.00	0.00	20.00	11.15	12.05

$Na_2MoO_2 \cdot 2H_2O$ 0.03 [56] was added at a volume of 50 mL. The initial pH was adjusted to 7 using 5 M NaOH, and the fermentation vessel was flushed with N_2 gas for 30 s, allowing for anaerobic conditions. Fermentation was carried out in a shaking water bath at a temperature of 37.5 °C and 80 rpm agitation for 72 h. Simultaneous saccharification and fermentation (SSF) H_2 production was carried out using a substrate concentration of 2.5 gVS, an inoculum concentration of 10% (v/v), and an initial pH of 6 due to the enzyme activity pH range. Cellulase from *Trichoderma reesei* was used at a dosage of 10 FPU/g in a 0.05 M citrate buffer. A volume of 50 mL sterile mineral salt solution was added, and the fermentation vessel was flushed with N_2 gas for 30 s. Fermentation was carried out at a temperature of 37.5 °C and 80 rpm agitation for 96 h.

Analytical methods

Compositional analysis

The solubilization of PMS was analyzed using the detergent fiber analysis technique described by Refs. [22,67]. PMS was subjected to a neutral detergent solution to solubilize and separate less digestible cell wall components such as cellulose, hemicellulose, and lignin from the digestible cellular components such as starch and sugar. Residual cell wall components were subsequently subjected to an acid detergent solution to solubilize and remove hemicellulose. The remaining components were treated with a $KMnO_4$:lignin buffer solution to solubilize and remove lignin. The final insoluble residue was then ashed to separate cellulose from insoluble ash [57].

Scanning electron microscopy (SEM)

Dried untreated and pretreated PMS samples were visualized using SEM. Samples were placed on adhesive carbon tape and gold sputter-coated using a Quorum 550x sputter coater. Samples were then viewed using a desktop Phenom Pharos scanning electron microscope at 10 kV.

Trace elements

Trace elements B, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Mo, Cd, Sn, Sb, Sr, Ba, Pb, and Hg were analyzed at the ICP-MS & XRF unit, Central Analytical Facilities at Stellenbosch University, South Africa. An Agilent 7900 quadrupole ICP-MS equipped with a High Matrix Introduction and a 4th generation Octopole Reaction system to remove polyatomic interferences from trace elements

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of interest. All trace elements were measured in He collision mode, while H_2 reaction gas was used for Se. Elements K, Na, Mg, Si, and P were analyzed using a Thermo ICAP 6200 ICP-AES with a charge injection device solid-state detector. Instrument calibration was carried out using a standard series prepared in 2% HNO_3, from NIST-traceable multi-element stock solutions and supra pure, double-distilled acids. ICP-MS standard for Hg was prepared in 2% HNO_3 and 2% HCl. Calibration standards and quality control standards were supplied by Inorganic Ventures (Inorganic Ventures, 300 Technology Drive, Christiansburg, VA 24073) and De Bruyn Spectroscopic Solutions (Bryanston, South Africa). Samples were prepared by microwave digestion (CEM MARS-5). Briefly, between 0.1 and 0.3 g sludge was digested using concentrated supra pure HNO_3 and HCl, cooled, and made up to 50 mL before analysis.

Fourier transform infrared (FTIR) analysis

To detect any changes observed in functional groups, sludge samples from each pretreatment step were analyzed by FTIR spectroscopy, using a PerkinElmer 100 (Waltham, MA, USA). Sludge samples were ground using spectroscopic grade KBr and further compressed to produce diameter pellets.

Hydrogen quantification

Biohydrogen volumes were recorded using the water displacement method [77]. The hydrogen fraction of the evolving gas was measured using a portable multi-gas analyzer (SKZ Industrial, Shandong, China). Cumulative hydrogen volume produced was calculated according to Eq. (1), where $V_{H,i}$ and $V_{H,i-1}$ are cumulative hydrogen gas volumes at the current (i) and previous (i-1) time intervals, $V_{G,i}$ and $V_{G,i-1}$ the total biogas volumes in the current and previous time intervals, $C_{H,i}$ and $C_{H,i-1}$ the fraction of hydrogen gas in the headspace of the fermentation vessel in the current and previous time intervals, and V_H the total volume of headspace in the fermentation vessel [55].

$$V_{H,i} = V_{H,i-1} + C_{H,i}\left(V_{G,i} - V_{G,i-1}\right) + V_H\left(C_{H,i} - C_{H,i-1}\right) \tag{1}$$

Results and discussions

Preliminary assessment

Initial surfactant-assisted GLD pretreatment screening was carried out in duplicate and used to determine the effect of combined surfactant and GLD pretreatment on releasing fermentable sugar from PMS. Reducing sugar yields of 0.24 g/g PMS were improved to 0.31 g/g PMS, corresponding to a 32% increase in reducing sugar release, from 11.91 to 15.70 g/L, when screened PMS was treated with Tween-80 and GLD. In comparison, pretreatment with GLD alone resulted in a reducing sugar yield of 0.28 g/g PMS, illustrating the enhanced effect of including a surfactant such as Tween-80 to the release of fermentable sugar.

Combined mixture and factorial model development

Experimental conditions and corresponding reducing sugar concentrations obtained are shown in Table 1. The experimental data were used to generate a Scheffe cubic x linear model for surfactant-assisted GLD pretreatment. Eq. (2) relates the concentration of reducing sugar obtained to the various input parameters:

$$\begin{aligned} \textit{Sugar release}\left(\frac{g}{L}\right) = {} & +12.61A + 12.99B - 0.041AB + 1.27AC \\ & + 0.052AD + 0.01867AE + 1.43BC - 0.9562BD + 0.0873BE \\ & - 2.9ABC + 2.43ABD - 2.59ABE + 33.87AB(A-B) \\ & + 47.63ABC(A-B) + 9.91ABD(A-B) + 35.97ABE(A-B) \end{aligned} \tag{2}$$

where A, B, C, D, and E represent PMS (%), GLD (%), surfactant concentration (%), time (min), and S:L (%), respectively.

This model's suitability was assessed using Analysis of Variance (ANOVA), seen in Table 2. A coefficient of determination (R^2) of 1 indicates a good fit [56]. The RSM model generated gave an R^2 value of 0.81, illustrating that the model could account for 81% of the variation observed in experimental data and could relate input parameters to the process response. A high F-value of 5.55, combined with a low p-value of <0.05 (0.0003), further illustrated the model's significance. Furthermore, each input parameter's suitability was determined by assessing the p-values, wherein input parameter significance was determined by exhibiting a p-value less than 0.05 [43]. Therefore interactions of input parameters AC, BC, BD, ABC exhibited a noted effect on the reducing sugar release for the model.

Effect of various process parameters on surfactant-assisted GLD pretreatment of PMS

Reducing sugar release values from various experimental runs are shown in Table 1, with corresponding response surface plots seen in Fig. 1.

The enhanced amenability of PMS to biological degradation, achieved after surfactant-assisted GLD pretreatment, was observed by an increase in the release of reducing sugar, from 10.35 to 14.95 g/L, corresponding to yields of 0.21–0.30 g/g. The highest release of reducing sugar was obtained under experimental conditions of 50% GLD, 50% PMS, 5% S:L, 5% Tween-80 conc. and 90 min (run 14); however, a reducing sugar release of 14.63 g/L (0.29 g/g) was obtained when a higher fraction of PMS was used, in combination with a higher S:L of 15% (20% GLD, 80% PMS, 15% S:L, 5% Tween-80 and 46.50 min – run 13). The positive effect of including a surfactant became more significant at higher substrate loadings, thus reducing the time required to obtain a similar yield from pretreatment [75]. The interactive effects of GLD to PMS and Tween-80 concentration, while process time and S:L were held at their median point, can be seen in Fig. 1a. At low GLD ratios of 20–50%, increasing the concentration of Tween-80 had a minimal effect on the release of reducing sugar, with a maximum release of 10 g/L reducing sugar at a Tween-80 concentration of between 1.5 and 2.5%. When the ratio of GLD was further increased from 50 to 80% and PMS was thus reduced from 50 to 20%, an increase in reducing sugar release, from 10 g/L to 15 g/L, was observed. A similar effect has been reported by Ref. [14]; where saturation of GLD in solution resulted in enhanced surface area accessibility, thus providing increased permeation efficiency during enzymatic hydrolysis.

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Table 2 – Analysis of Variance (ANOVA) of the developed surfactant-assisted GLD pretreatment model.

Factor	Sum of squares	df	Mean square	F-value	p-value (prob. > F)	
Model	32.56	15	2.17	5.55	0.0003	Significant
Linear	0.2684	1	0.2684	0.6858	0.4174	
AB	0.0007	1	0.0007	0.0019	0.9659	
AC	11.17	1	11.17	28.54	<0.0001	
AD	0.0188	1	0.0188	0.0481	0.8287	
AE	0.2423	1	0.2423	0.6190	0.4406	
BC	8.40	1	8.40	21.45	0.0002	
BD	3.32	1	3.32	8.48	0.0086	
BE	0.0260	1	0.0260	0.0664	0.7993	
ABC	1.94	1	1.94	4.96	0.0376	
ABD	1.51	1	1.51	3.87	0.0633	
ABE	1.68	1	1.68	4.30	0.0514	
AB (A-B)	1.73	1	1.73	4.42	0.0484	
ABC (A-B)	1.77	1	1.77	4.52	0.0461	
ABD (A-B)	0.1063	1	0.1063	0.2716	0.6080	
ABE (A-B)	1.20	1	1.20	3.07	0.0950	
Residual	7.83	20	0.3914	–	–	
Lack of fit	5.39	17	0.3168	0.3890	0.9117	Not significant
Pure error	2.44	3	0.8144	–	–	
Cor total	40.39	35	–	–	–	

A = PMS (%), B = GLD (%), C = Surfactant conc. (%), D = Time (min), E = S:L (%).

At higher ratios of GLD (50–80%), the enhancement of enzymatic hydrolysis by increasing concentrations of Tween-80 (from 2.5 to 5%) was highlighted, increasing the reducing sugar released from 15 g/L to more than 20 g/L. This may be due to the effect of Tween-80 addition, which has been reported to reduce adsorption of cellulase onto lignin and increase desorption of cellulase into the PMS fibers, thus improving the efficiency of enzymatic hydrolysis of pretreated PMS fibers [75]. The large increase in reducing sugar release, from <10 g/L (yield of 0.20 g/g) to 22 g/L (0.44 g/g) at an increased concentration of Tween-80 (5%), illustrated the significant impact that the addition of a surfactant had on reducing sugar release after enzymatic hydrolysis of PMS. Furthermore, low PMS concentrations (20–35%) in combination with high surfactant concentration (5%) suggest that the surfactant must be present in saturating quantities, even after pretreatment, to adsorb onto cellulose during enzymatic hydrolysis effectively [19]. Reported several ways in which nonionic surfactants positively affect cellulosic enzyme hydrolysis; a) increasing the surface area of cellulose, b) removing lignin which is inhibitory to enzyme hydrolysis, and c) increasing the stability of enzymes during enzyme hydrolysis.

Fig. 1b shows the interactive effects of GLD to PMS and pretreatment time, while Tween-80 concentration and S:L were held at their median points of 2.5% and 10%, respectively. At a low GLD ratio (20%) and high PMS ratio (80%), an increase in pretreatment time, from 20 to 60 min, released a reducing sugar concentration of 12 g/L. Increasing the GLD and reducing the PMS concentration ratios from 20:80 to 35:65 resulted in a lower reducing sugar release of 10 g/L – and increasing pretreatment time from 10 to 90 min interestingly led to a broader range of GLD to PMS ratios which produced lower reducing sugar. This negative effect was overcome by further increasing the ratio of GLD to PMS from 35:65 to 65:35 to obtain a maximum of 16 g/L reducing sugar – due to a higher GLD saturation level. The effect of pretreatment time can also be observed to be insignificant at all GLD to PMS ratios, with only a slight increase in its effect at higher GLD to PMS ratios and higher pretreatment times. The interactive effects of GLD to PMS (%) and S:L (%), while Tween-80 concentration and pretreatment time were held at their median points of 2.5% and 55 min, respectively, can be observed in Fig. 1c. A low S:L ratio of 5% exhibited a slight effect on reducing sugar release, regardless of the ratio of GLD to PMS implemented. Upon an increase in S:L, from 9 to 15%, a more significant effect of variation in GLD to PMS was observed. Increasing the concentration of PMS (thus reducing the concentration of GLD) displayed a severe negative impact on the release of reducing sugar, resulting in a release of no more than 6 g/L reducing sugar at an elevated S:L ratio of 15% and a GLD to PMS ratio of 35:65. To overcome the negative interaction of a high PMS concentration and high S:L, an increased GLD concentration significantly increased, reducing sugar release to 15 g/L at an S:L of 9% and 19 g/L at an S:L of 15%. The negative impact of increased PMS within the pretreatment system may be due to this substrate's high water holding capacity [33]. [64] highlighted the significance of water in the hydrolysis of lignocellulosic biomass, where water at increased temperature and pressure supports cellulose and hemicellulose hydrolysis.

Model validation

Based on model predictions, optimal pretreatment conditions of 56% GLD, 44% PMS, 4.5% Tween-80, 60 min heating time, and S:L of 9.5% should result in a reducing sugar release of 16.70 g/L (0.334 g/g). Experimental validation of the developed surfactant-assisted GLD pretreatment model was carried out in triplicate to determine the model's predictive accuracy, generating an optimal reducing sugar release of 16.38 g/L

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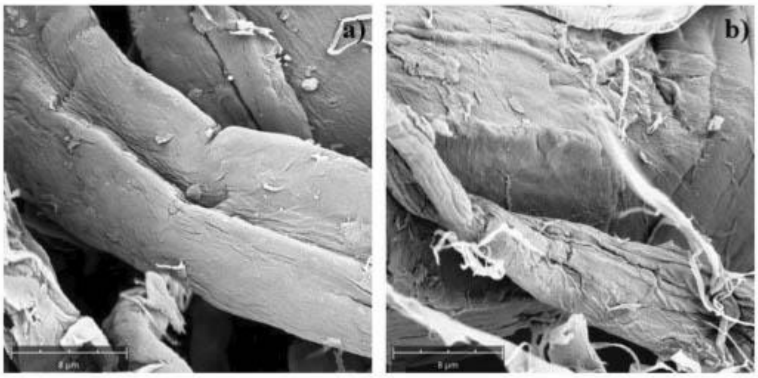

Fig. 2 – SEM images of PMS before and after surfactant-assisted GLD pretreatment. a) untreated PMS and b) optimally pretreated PMS.

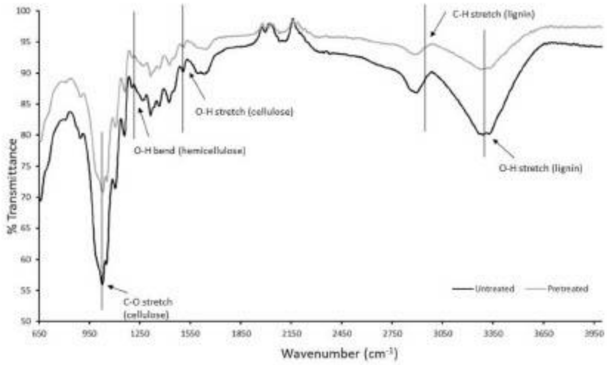

Fig. 3 – FTIR spectra of untreated and pretreated PMS under optimal conditions.

glycosidic linkages illustrate the effect of pretreatment on cellulose availability and correlate with the SEM images obtained [14]. An increase in percentage transmittance at bands observed at 2907 to 2996 cm^{-1} (C–H stretching in lignin) and 3000 to 3700 cm^{-1} (O–H stretching in lignin) for the pretreated PMS can be attributed to the removal of lignin during pretreatment [2,68].

Effect of surfactant-assisted GLD pretreatment on the elemental composition of PMS

ICP analysis was conducted on PMS samples after each step of the pretreatment process to illustrate the effect of each step on the elemental composition of PMS (Table 4). Heavy metals are often found in black liquor and thus in wastewater generated by the pulp and paper industry [1]. Detected in quantities below 1 mg/kg were predominantly heavy metals Arsenic (As), Selenium (Se), Cadmium (Cd), Antimony (Sb), and Mercury (Hg). Due to its similarity with phosphate, arsenic is known to be the most toxic heavy metal to humans - found in water, soil, and air [15,28] and has no biological role (non-essential metal). It occurs in four oxidation states, i.e., arsenate and arsenite (inorganic) and elemental arsenic and arsenide (organic) [15,28], with arsenite displaying the most toxicity by binding with sulfur thiolates, forming complexes with glutathione, thus generating free radicals and inhibiting oxidative metabolism by strongly binding pyruvate dehydrogenase [15]. Non-essential metal toxicity in microorganisms has been reported by Ref. [15] to occur by the displacement of essential metals from their binding sites or by ligand interactions. Microorganisms in particular display varying levels of arsenic resistance, ranging from complete inhibition at 24.70 mg/L [60] to 6000 mg/kg; however, the addition of phosphate has been shown to reduce the toxicity and inhibitory effect of arsenic [13]. A reduction in arsenic concentration illustrates the removal or reduction of arsenic in PMS,

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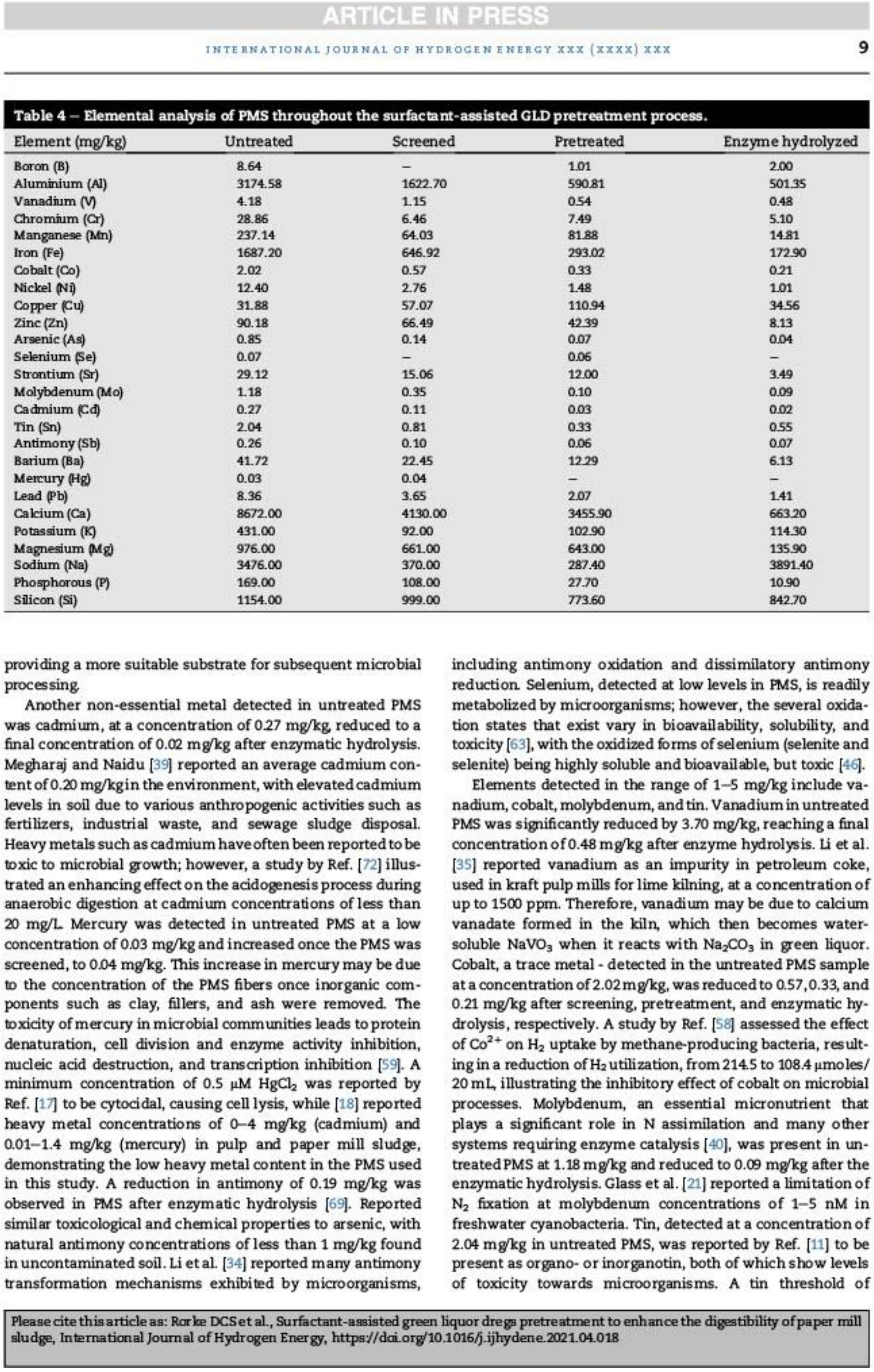

Table 4 – Elemental analysis of PMS throughout the surfactant-assisted GLD pretreatment process.

Element (mg/kg)	Untreated	Screened	Pretreated	Enzyme hydrolyzed
Boron (B)	8.64	–	1.01	2.00
Aluminium (Al)	3174.58	1622.70	590.81	501.35
Vanadium (V)	4.18	1.15	0.54	0.48
Chromium (Cr)	28.86	6.46	7.49	5.10
Manganese (Mn)	237.14	64.03	81.88	14.81
Iron (Fe)	1687.20	646.92	293.02	172.90
Cobalt (Co)	2.02	0.57	0.33	0.21
Nickel (Ni)	12.40	2.76	1.48	1.01
Copper (Cu)	31.88	57.07	110.94	34.56
Zinc (Zn)	90.18	66.49	42.39	8.13
Arsenic (As)	0.85	0.14	0.07	0.04
Selenium (Se)	0.07	–	0.06	–
Strontium (Sr)	29.12	15.06	12.00	3.49
Molybdenum (Mo)	1.18	0.35	0.10	0.09
Cadmium (Cd)	0.27	0.11	0.03	0.02
Tin (Sn)	2.04	0.81	0.33	0.55
Antimony (Sb)	0.26	0.10	0.06	0.07
Barium (Ba)	41.72	22.45	12.29	6.13
Mercury (Hg)	0.03	0.04	–	–
Lead (Pb)	8.36	3.65	2.07	1.41
Calcium (Ca)	8672.00	4130.00	3455.90	663.20
Potassium (K)	431.00	92.00	102.90	114.30
Magnesium (Mg)	976.00	661.00	643.00	135.90
Sodium (Na)	3476.00	370.00	287.40	3891.40
Phosphorous (P)	169.00	108.00	27.70	10.90
Silicon (Si)	1154.00	999.00	773.60	842.70

providing a more suitable substrate for subsequent microbial processing.

Another non-essential metal detected in untreated PMS was cadmium, at a concentration of 0.27 mg/kg, reduced to a final concentration of 0.02 mg/kg after enzymatic hydrolysis. Megharaj and Naidu [39] reported an average cadmium content of 0.20 mg/kg in the environment, with elevated cadmium levels in soil due to various anthropogenic activities such as fertilizers, industrial waste, and sewage sludge disposal. Heavy metals such as cadmium have often been reported to be toxic to microbial growth; however, a study by Ref. [72] illustrated an enhancing effect on the acidogenesis process during anaerobic digestion at cadmium concentrations of less than 20 mg/L. Mercury was detected in untreated PMS at a low concentration of 0.03 mg/kg and increased once the PMS was screened, to 0.04 mg/kg. This increase in mercury may be due to the concentration of the PMS fibers once inorganic components such as clay, fillers, and ash were removed. The toxicity of mercury in microbial communities leads to protein denaturation, cell division and enzyme activity inhibition, nucleic acid destruction, and transcription inhibition [59]. A minimum concentration of 0.5 μM $HgCl_2$ was reported by Ref. [17] to be cytocidal, causing cell lysis, while [18] reported heavy metal concentrations of 0–4 mg/kg (cadmium) and 0.01–1.4 mg/kg (mercury) in pulp and paper mill sludge, demonstrating the low heavy metal content in the PMS used in this study. A reduction in antimony of 0.19 mg/kg was observed in PMS after enzymatic hydrolysis [69]. Reported similar toxicological and chemical properties to arsenic, with natural antimony concentrations of less than 1 mg/kg found in uncontaminated soil. Li et al. [34] reported many antimony transformation mechanisms exhibited by microorganisms, including antimony oxidation and dissimilatory antimony reduction. Selenium, detected at low levels in PMS, is readily metabolized by microorganisms; however, the several oxidation states that exist vary in bioavailability, solubility, and toxicity [63], with the oxidized forms of selenium (selenite and selenite) being highly soluble and bioavailable, but toxic [46].

Elements detected in the range of 1–5 mg/kg include vanadium, cobalt, molybdenum, and tin. Vanadium in untreated PMS was significantly reduced by 3.70 mg/kg, reaching a final concentration of 0.48 mg/kg after enzyme hydrolysis. Li et al. [35] reported vanadium as an impurity in petroleum coke, used in kraft pulp mills for lime kilning, at a concentration of up to 1500 ppm. Therefore, vanadium may be due to calcium vanadate formed in the kiln, which then becomes water-soluble $NaVO_3$ when it reacts with Na_2CO_3 in green liquor. Cobalt, a trace metal - detected in the untreated PMS sample at a concentration of 2.02 mg/kg, was reduced to 0.57, 0.33, and 0.21 mg/kg after screening, pretreatment, and enzymatic hydrolysis, respectively. A study by Ref. [58] assessed the effect of Co^{2+} on H_2 uptake by methane-producing bacteria, resulting in a reduction of H_2 utilization, from 214.5 to 108.4 μmoles/20 mL, illustrating the inhibitory effect of cobalt on microbial processes. Molybdenum, an essential micronutrient that plays a significant role in N assimilation and many other systems requiring enzyme catalysis [40], was present in untreated PMS at 1.18 mg/kg and reduced to 0.09 mg/kg after the enzymatic hydrolysis. Glass et al. [21] reported a limitation of N_2 fixation at molybdenum concentrations of 1–5 nM in freshwater cyanobacteria. Tin, detected at a concentration of 2.04 mg/kg in untreated PMS, was reported by Ref. [11] to be present as organo- or inorganotin, both of which show levels of toxicity towards microorganisms. A tin threshold of

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750–1000 mg/kg was reported by Ref. [37] in a study that tested the influence of tin ions on the development and enzymatic activity of fungal strains; however, there is limited information on the effects of tin on other microorganisms.

Detected at concentrations of 8.64 and 8.36 mg/kg in untreated PMS were boron and lead. Boron was subsequently reduced to 2 mg/kg after enzymatic hydrolysis of PMS. Boron (present in the environment at 0.1–10 mg/L) is a micronutrient essential for N fixation in bacteria but exhibits an inhibitory effect on microbial growth at high concentrations [42]. A threshold of 3.80–47.60 mg/mL of boron compounds such as boric acid and borax was determined by Ref. [71]. Lead detected in the untreated sample was reduced to 3.65 mg/kg, illustrating less efficient removal after screening than the reduction observed for boron. Pretreatment reduced the lead concentration by 1.58 mg/kg, with further removal of 0.66 mg/kg after enzyme hydrolysis. Lead is one of the major pollutants, reaching up to 10,000 mg/kg in industrial areas and 200–250 mg/L in industrial wastewater, which is well above the water quality standard of 0.05–0.10 mg/L. Lead content of 1–83 mg/kg was reported by Ref. [18] in pulp and paper mill sludge. Lead usually binds to clay minerals and complex organic compounds, consequently lowering the lead fraction, which is bioavailable; however, lead is toxic, even at low concentrations [29]. Despite this, numerous microorganisms have developed mechanisms allowing their survival in environments of high lead concentrations.

Elements detected in the untreated PMS at concentrations between 10 and 30 mg/kg included chromium, strontium, and nickel. Chromium was significantly reduced from 28.86 to 5.10 mg/kg after enzyme hydrolysis. Heavy metals such as chromium are often present in black liquor, which can be recycled into green liquor, from which GLD is obtained [1]. The slight increase in chromium observed in the pretreated PMS sample may be attributed to the possible chromium presence in GLD. Chromium exhibits the most toxicity based on its oxidation state, with Cr(VI) being highly toxic. The reduction of Cr(VI) to lower oxidation states results in free radical production, which causes its toxicity. Chromium levels of 80 mg/kg in paper mill effluents have been reported by Ref. [8]; which is significantly higher than the observed 28.86 mg/kg in untreated PMS. Strontium, detected at a concentration of 29.12 mg/kg in untreated PMS, is a soft metal, similar to lead, but possesses similar chemistry to that of calcium, readily reacting with halogens, oxygen, and sulfur [50]. The strontium concentration was significantly reduced by 25.63 mg/kg after hydrolysis. Due to various uptake mechanisms available for calcium, strontium can accumulate within cells and exhibit toxicity at higher concentrations [50]. Nickel was detected in the untreated PMS at a 12.40 mg/kg concentration and was reduced to 1.01 mg/kg after screening, pretreatment, and enzyme hydrolysis. Chromium and nickel concentrations in pulp and paper mill sludge of 1–44 and 1–32 mg/kg, respectively, were reported by Ref. [18].

Elements detected in the untreated PMS within the range of 30–100 mg/kg were trace metals copper (31.88 mg/kg), zinc (90.18 mg/kg) and barium (41.72 mg/kg). Trace metals are beneficial to maintaining a diverse, healthy microbial community and are often added to wastewater to assist chemical oxygen demand (COD) removal. However, the addition must be applied with caution as high levels of these elements can lead to growth inhibition in various microbial processes [5]. The increase in copper observed after screening and pretreatment may be due to copper in distilled water [12] used in both screening and pretreatment of PMS. Copper was then significantly reduced to 34.56 mg/kg after enzyme hydrolysis and may be linked to a type II copper site in GH61 proteins that play a significant role in enhancing cellulose degradation in *Trichoderma reesei* [16]. The presence of zinc in untreated PMS was reduced by 82.05 mg/kg after screening, pretreatment, and enzyme hydrolysis, resulting in a final concentration of 8.13 mg/kg. Additionally, barium concentrations were reduced to 6.13 mg/kg after enzyme hydrolysis. The use of barium as a supplemental additive has been shown to increase the activity of extracellular hydrolytic enzymes and may therefore contribute to the hydrolysis of cellulose in PMS [45].

Elements determined in untreated PMS in the range of 100–300 mg/kg were minerals manganese and phosphorous at concentrations of 237.14 and 169 mg/kg, which were reduced to 14.81 and 10.90 mg/kg, respectively, after enzyme hydrolysis. The minerals potassium and magnesium were reduced to 114.30 and 135.90 mg/kg, respectively, after enzyme hydrolysis. The minerals present in enzymatically hydrolyzed PMS can positively impact subsequent microbial fermentation processing of PMS due to their significance in various cellular mechanisms. Elements detected in the range of 1–10 g/kg in untreated PMS were aluminium (3174.58 mg/kg), iron (1687.20 mg/kg), silicon (1154 mg/kg) and calcium (8672 mg/kg) and exhibited reductions of 2673.28, 1514.30, 311.30 and 8008.80 mg/kg respectively after enzyme hydrolysis. Greaves [26] reported a toxicity of manganese > magnesium > iron > calcium > sodium > potassium, with divalent cations exhibiting higher toxicity than monovalent cations in soil microorganisms. Interestingly, a sodium increase from 287.40 after pretreatment to 3891.40 mg/kg after enzyme hydrolysis was observed, which may be due to the presence of sodium citrate in the citrate buffer used during enzymatic hydrolysis.

Table 5 – Hydrogen yields obtained after each pretreatment step of the developed surfactant-assisted GLD model.

PMS pretreatment applied	Hydrogen yield (mL/g)	Improvement (%)
Untreated	–	–
Untreated + EH	2.49	–
Screened	–	–
Screened + EH	2.53	1.61
Screened + SAGLDP	2.64	6.02
Screened + SAGLDP + EH	2.73	9.64
SSF H_2 production		Improvement (%)[a]
Untreated	2.94	18.07
Screened	2.89	14.22
Screened + SAGLDP	3.72	36.26

EH: Enzymatic hydrolysis; SAGLDP: surfactant-assisted GLD pretreatment; SSF: simultaneous saccharification and fermentation.

[a] Values based on similar pretreatment applied, with EH conducted separately.

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Stage-gate analysis of pretreatment protocol for the production of hydrogen

Hydrogen yields obtained after preliminary dark fermentation on PMS samples at each phase of the established surfactant-assisted GLD pretreatment are shown in Table 5. It was observed that a 0.15 mL/g (6%) hydrogen yield increase was obtained when untreated PMS was screened and pretreated. A further 9.64% improvement was achieved when screened, and pretreated PMS was enzymatically hydrolyzed before hydrogen production. Furthermore, SSF H_2 production on screened and pretreated PMS resulted in a 36.26% yield improvement compared to the hydrogen yield obtained when enzyme hydrolysis was performed in a separate step before hydrogen production. Moreno-Dávila et al. [44] reported optimal SSF conditions of pH 4, 70 FPU enzyme loading and 45 °C, using Celluclast®, obtaining a hydrogen yield of 55 844 mmol/h.gSV from H_2SO_4 pretreated paper industry waste.

These findings illustrate the potential of screened and optimally GLD pretreated PMS for subsequent use in SSF H_2 production. Including GLD as a pretreatment agent will reduce the energy input and cost required to pretreat lignocellulosic wastes such as PMS while simultaneously providing an alternative use for the waste GLD generated by the pulp and paper industry. Additionally, the use of waste such as GLD as a pretreatment agent for the paper industry's waste PMS would be simple to incorporate into paper mills as the infrastructure required to accommodate these wastes and convert lignocellulose such as PMS at large volumes already exists. Both the pretreatment agent and the lignocellulosic material to be treated originate from the same industry, reducing possible logistical costs. As a result, pulp and paper mills could include biogas production plants within their current infrastructure, with the intention of waste valorization, which will improve the economics and profitability of renewable energy production. GLD is a waste that is often landfilled, negatively affecting the pulp and paper industry's economics and the environment in which it is disposed of [14], thus illustrating the potential benefit of redirecting this waste to an avenue of better use.

Conclusion

This study focused on optimizing a novel surfactant-assisted GLD pretreatment and assessed the effect of this pretreatment protocol on the composition and, thus, amenability to microbial processing of PMS. Optimized pretreatment conditions resulted in a reducing sugar release of 16.38 g/L (0.328 g/g). The effect of the pretreatment protocol was assessed by SHF and SSF H_2 production, giving a hydrogen yield of 2.73 and 3.72 mL/g, respectively, illustrating the positive impact of including an enzymatic hydrolysis phase within the production of hydrogen. This study developed an efficient surfactant-assisted GLD pretreatment protocol to enhance the amenability of PMS, which is often regarded as a material that is difficult to degrade or dispose of, using low cost, readily available waste from the same industry as the lignocellulosic material itself. This will ultimately increase the practicality of pretreatment implementation while reducing process costs and energy requirements.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijhydene.2021.04.018.

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CONFERENCE ABSTRACTS

Valorisation of Pulp and Paper Mill Sludge for Biofuel Production

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The large wastewater treatment system of the pulp and paper industry leads to the generation of high volumes of waste sludge, the disposal of which poses significant threats to environmental safety and process economics. This study investigates the potential of this waste to be used as a raw material for biogas production. The biomethane potential (BMP) of both raw and heat-treated mill sludge is assessed under mono-digestion as well as co-digestion conditions.

BMP assays were conducted over a duration of 40 days, using cow dung as a co-digestion substrate. Additionally, characterisation was carried out on the mill sludge to further determine the potential of the substrate to support the growth and proliferation of methane producers.

Pyrolysis GC-MS characterisation showed significant levels of acetic acid, fluoroacetylene, hydroxyacetone, pyruvaldehyde and glucose within the mill sludge, while a carbon-to-nitrogen ratio (C:N) of 910 was determined. Furthermore, HPLC analysis showed a mill sludge carbohydrate content profile of 0.70, 1.20, 59.20, 6.00 and 7.20 % of arabinose, galactose, glucose, xylose and mannose, respectively, while a lignin content of 28 % was observed.

BMP assays showed very low methane potentials, with raw mill sludge (maximum of 1.29 ml), while a significantly lower methane output of 0.98 ml was obtained from heat-treated mill sludge. In contrast, although a slightly lower methane volume of 1.24 ml was obtained when raw sludge was co-digested with cow dung, a 50% increase in the CH_4 fraction was observed.

These findings illustrate the need to make use of anaerobic co-digestion in order to enhance the feasibility of using pulp and paper mill sludge for biogas production.